

THE EARLY DEVELOPMENT
OF
AMBLYSTOMA

WITH OBSERVATIONS ON SOME OTHER
VERTEBRATES

A THESIS
FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
PRESENTED TO THE
BIOLOGICAL FACULTY OF THE UNIVERSITY OF CHICAGO
DEC. 15, 1894

BY
ALBERT CHAUNCEY EYCLES HYMER

Reprinted from JOURNAL OF MORPHOLOGY, Vol. X., No. 2

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ALBERT C. EYCLESHYMER.

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THE following work was begun at Clark University in the autumn of 1892, and continued during the succeeding summer in the Marine Biological Laboratory at Woods Holl. Since that time it has been carried on in the Zoölogical Laboratory of the University of Chicago.

An attempt to express my gratitude to Professor C. O. Whitman, at whose suggestion the study was undertaken, and whose criticism has ever been a source of inspiration, would indeed prove futile and sound extravagant.

The intention was at first to study certain phases in the ontogeny of *Amblystoma punctatum* (Baird), but the work has been gradually extended so as to include a comparison of particular features of development in *Necturus*, *Rana*, *Petromyzon*, *Amiurus*, *Lophius*, and *Coregonus*.

I am indebted to Professor C. O. Whitman for *Necturus* material, to Dr. W. M. Wheeler for *Rana palustris*, to Dr. H. P. Johnson for *Petromyzon*, to Mr. A. D. Mead for *Lophius*, and to Professor J. E. Reighard for *Amblystoma tigrinum*.

Habits, Oviposition, etc. — *Amblystoma* is probably the most widely distributed of all the Urodela, being found in nearly every state of the Union, as well as in Canada and Mexico. For its systematic position the reader is referred to the works of Cope, Hay, Garman, and others.

For observing the aquatic habits of these animals early spring is most favorable, since at this time they migrate to the ponds where the eggs are deposited. The time of their appearance varies in different localities. For the past four or five years, in the vicinity of Ann Arbor, Mich., they have appeared regularly on or about the 22d of March. At Worcester, Mass., I found eggs, presumably of *Amblystoma punctatum*, on April 6, 1892, while in eastern Wisconsin the animals were observed April 15, 1891.

The migration to the breeding grounds probably occurs at night, since they are rarely seen on land during the day. Their abrupt appearance in large numbers has often been noted, and at this time they may be easily captured; their appearance is no less sudden than their departure,—in two or three days they become extremely scarce and soon disappear.

They seem to be carnivorous; dissections of the alimentary tract indicate that their food is largely Crustacea. The young eagerly devour pieces of meat or insect larvae. In the absence of other food they prey upon each other.

The female is very choice in selecting a place to deposit her eggs, striving to obtain a position where she can rest upon a small twig or blade of grass and at the same time keep her nostrils above the water. She then clasps the twig between her posterior limbs and presses it tightly against the cloaca. The eggs escape singly, and adhere to the object on which they are deposited. The process may be continuous, covering a period of one or two hours, or at irregular intervals, lasting from a few hours to a day. The eggs may be deposited singly or in masses of a hundred or more.

In some localities the water is so turbid that considerable difficulty is experienced in obtaining the eggs. By placing a number of bushes in the pond the animals were attracted; the following morning the bushes were raised and a part of the eggs collected. In this way freshly deposited eggs were readily obtained.

The fresh eggs are so closely crowded together and firmly held in place by the gelatinous envelopes that they can only be removed with difficulty; after remaining in the water for a few hours the envelopes swell and the removal is easily effected. At this time the egg presents the appearance indicated in Pl. XVIII, Fig. 1, being enclosed by three concentric envelopes. The outermost, by means of which the eggs become attached to the object on which they are deposited, is transparent and structureless. Numbers of minute algae are often found in this membrane; but their presence in the internal envelope, as noted by Orr (**88**), has not been observed. The middle envelope presents a more irregular outline. When

treated with Schneider's acetic carmine a finely punctated appearance is revealed, probably due to the presence of minute pores. The inner or vitelline membrane is always so closely applied to the egg that it is not easily detected.

I. CLEAVAGE TO GASTRULATION.

I. METHODS.

To plainly observe the phases of cleavage it is necessary to remove the envelopes. This is best accomplished by seizing the mass with forceps and at the same time piercing and snipping the envelopes with spring-scissors, allowing the eggs to fall to the bottom of the dish. It is often desirable to fix the eggs before the laboratory can be reached. I found it a good plan to carry a jar of Perényi's fluid and to place the eggs at once in this; when the laboratory is reached the envelopes may be removed, or the mass transferred to 70% alcohol and preserved indefinitely. When ready for study they are passed through 50% alcohol to water, and the envelopes removed by using a weak solution of hypochlorite of sodium (eau de Labarraque).

In the study of the living egg a plane mirror was placed beneath the watch-glass containing the egg as suggested by Pflüger ('83). This is of great assistance, since the changes going on at either pole may be observed without disturbing the position of the egg.

Various reagents have been employed in fixing, among these Perényi's fluid stands first for general histology. Picro-acetic gave excellent results, especially in cleavage. Chrom-formic and chrom-osmo-acetic were also used.

Among the many stains used, I especially recommend Czokor's alum-cochineal, Mayer's alcoholic-carmine, and Grübler's borax-carmine. I have used the Biondi-Ehrlich mixture, also borax-carmine and nigrosine for double staining with excellent results. The staining has been tried both *in toto* and in section, the latter invariably giving the better differentiation.

In imbedding both the celloidin and paraffine methods have been employed. Owing to the crumbling of the yolk and the

shrinkage of the eggs, often producing artificial delaminations and cavities, it was found necessary to discard the latter. Serial sections by the method which I have described in another place ('92) have been used for the most part. A method which I found very instructive was to imbed several eggs without reference to planes. This obviously necessitated reconstructions.

In reconstruction the wax plate method gave most satisfactory results. I have also profited by camera lucida drawings on panes of glass; the outlines of the various structures being drawn in water colors. By combining these, a graphic representation of the embryo is obtained. Projections on ruled paper have also assisted in gaining an idea of the structures.

In the present paper I shall omit entirely the discussion of the phenomena of maturation and fertilization, since these subjects have been so thoroughly studied by Jordan ('93) in the Newt and Fick ('93) in the Axolotl.

2. THE UNSEGMENTED OVUM.

The fertilized ovum of *Amblystoma* is nearly spherical, being slightly flattened at the pigmented pole and measuring about 2 mm. in diameter. The upper portion presents a deep brown color (Pl. XVIII, Fig. 1), which in a zone just above the equator changes quite abruptly to a lighter shade, and fades away into a faint yellow at the opposite pole. A marked difference in the density of pigment on the opposite side of the upper hemisphere is often noticeable. While this difference is by no means constant, I believe it of sufficient importance to be kept in mind with reference to the orientation of the embryo.

If the egg be examined a few hours after deposit it presents the appearance shown in Fig. 2. There is a very conspicuous saucer-shaped depression surrounded by a zone in which the pigment is less dense than in other portions; to this area various names have been applied, as, "Keimpunkt," "fovea germinativa," "trou vitellin," "light spot," *etc.* Slight magnification reveals at its center a small pit in which the first polar globule lies.

The superior pole some hours later (Fig. 3) shows no trace of the saucer-shaped depression. The pit at its center has elongated as indicated in Figs. 3 and 4. In the meantime a second polar body has appeared. The elongation or slit-like extension of the pit was also observed in the eggs of *Rana*.

I have sectioned a number of eggs at this stage to determine what relation, if any, the pigmented path of the spermatozoon bears to this slit, but no fixed relation has been discovered.

In eggs where the pigment is eccentric I have likewise endeavored to determine what relation a line drawn through the centers of the lightest and darkest portions bore to this slit, but efforts in this direction were also fruitless.

3. CLEAVAGE OF AMBLYSTOMA.¹

In the following pages I shall describe the successive stages of a particular egg which presents a fairly typical cleavage, recording in each case the variations most frequently observed in the twenty to thirty living eggs studied; these variations being confirmed by a study of hardened material.

The term cleavage, as here used, covers the whole period of cell-formation up to the time of gastrulation.

First cleavage. — The appearance of the first furrow is foreshadowed by a flattening at the superior pole, which soon changes to a furrow. The progress of this furrow over the dark hemisphere is rapid and continuous, covering a period of less than 15 min. As the furrow passes toward the opposite pole its rate of progress constantly decreases, and it is 1 hr. and 53 min. (Fig. 13) before the entire periphery of the egg is encircled by a continuous furrow (Fig. 5). The furrow is vertical, dividing the egg, as a rule, into two nearly equal parts. Although I have often observed the progress of the ends of the furrow, I have never found any indication of an alternate widening and narrowing of the opposite ends as recorded by von Baer ('34).

An interesting phenomenon which occurs during the progress of the furrows is the formation of the so-called "Faltenkränzen." Whether they are to be regarded with Reichert ('41) and others

¹ Some of the facts presented in this portion of the paper are the results of a joint study with Dr. E. O. Jordan ('94). I gladly acknowledge my indebtedness to him.

as a consequence of the contraction of the yolk, or are to be considered with Max Schultze ('63) as an expression of a radial arrangement of the yolk particles, I am unable to determine.

The origin of the furrow with reference to the slit-like orifice already described has been carefully studied. Unless special attention be directed to this, one is very apt to infer that it is the beginning of the first cleavage. More careful study shows the two are entirely independent, and that no fixed relation exists between them. In *Petromyzon*, Calberla ('78) believed the two to coincide.

It is by no means always the case that the first furrow divides the egg into two equal parts; it may depart so far from a meridional that the segments formed are quite unequal, as observed in the Frog by Prévost and Dumas ('24), Rusconi ('36), Newport ('51), and others. Neither do the ends of the furrow always unite with each other in a straight line, but are often joined in such a manner that an obtuse angle is formed.

Sections of this stage show that the yolk is highly differentiated, the granules at the vegetative pole being largest, and gradually decreasing in size toward the animal pole until the upper portion is constituted of a finely granular protoplasm.

Second cleavage. — In many cases before the ends of the first furrow have reached the inferior pole, the appearance of a second set is foretold by the rapid closing of the groove at the dark pole; this continues until there is but a faint indication of its presence.

The second furrows begin at the first, and extend in either direction at right angles to it (Fig. 6). Their progress is in all essentials like that of the first, and in 1 hr. and 46 min. after the time of appearance they have reached the opposite pole.

Variations in the formation of the second set of furrows are not uncommon. The point of origin may be at the pole or at a greater or less distance from it. Newport ('51) observed this eccentric origin, and believed that the second furrows formed segments which on one side were always larger than those on the other.

Instead of a right angle, acute and obtuse angles may arise in such a manner that they form an X, as figured by Prévost

and Dumas ('24). Again, the furrows may not arise in the same locality, in which case the two are united by the short piece of the first furrow intervening, to which the designation "cross-furrow" has been applied.

These furrows do not always begin at the same time ; one may precede the other by a considerable interval. At the vegetative pole many variations are likewise found ; often the two join to form a continuous line running at right angles to the first, again forming acute and obtuse angles.

In the axial line where the four quadrants of the egg join, along a region just above the center of the egg, sections show an open space, which is the first indication of the segmentation cavity.

Third cleavage.—In 1 hr. 45 min. after the appearance of the second set of furrows, and even before they have reached their destination, there are indications of the first horizontal division at or near the limits of the dark hemisphere (Fig. 7). In the particular egg from which the diagrams are made, quadrant *b* (Fig. 14) was the first to divide, the furrow started from the second vertical (indicated in the figure by a dotted line) and progressed from left to right. In 4 min. it reached the first vertical.

The next division occurred in the adjacent cell *d* a few moments later, extended in the same direction, and reached its destination in about the same time ; 3 min. later a third furrow, starting from the first vertical, appeared in cell *a*, and progressed in the same direction as the two preceding. Its rate was more rapid, however, reaching the point at which the first terminated in less than 2 min. Before this furrow was complete, cell *c* divided from right to left. The entire time occupied in the formation of this set of furrows is extremely brief as compared with the preceding cleavage.

In point of origin and direction of progress many variations occur. They may depart from a common point at either the first or second vertical. In many cases they appear successively in the different quadrants, following the direction of the hands of a clock, again passing in precisely the opposite direction.

It often happens that some of the furrows, instead of being horizontal, are more or less inclined, at times passing into the vertical. Jordan and myself ('92) described such variations in the Newt, Frog, and Amblystoma. Morgan and Tsuda ('94) have since recorded similar variations in the Frog's egg.

Fourth cleavage. — About 1 hr. and 50 min. later, the fourth set of furrows appear. In the majority of cases they are vertical, as shown in Fig. 8. They often all depart from one or the other of the first two verticals; sometimes, however, some depart from one, while the rest depart from the other, as shown in Fig. 15. In short, this set offers so many variations that a type is no longer recognizable. In the egg represented (Fig. 15) the first of this set appeared in quadrant *b* at the first vertical. The next was formed in the adjacent quadrant *a* 2 min. later, its point of origin being at one of the second verticals. The third furrow, starting from the first vertical, passed across quadrant *d* 1 min. later; the division of *c* followed 1 min. later, and this set passed toward the lower pole, which point some reached, while others turned aside and united to form a common furrow.

The entire set of verticals may depart from a common point at the superior pole and cut each quadrant into two approximately equal portions, in which case a radially symmetrical condition obtains. It was this condition which von Baer ('34) observed in *Rana* and considered as a type. Yet Rauber ('83) denies this a typical value, and states that he has not seen a single egg where such was the case.

Another condition often observed is one in which the position of the furrows in hemisphere *ab* (Fig. 15) would be similar to that in *cd*; in this case there is a distinctly bilateral appearance. The furrows often run nearly parallel to the first vertical, and again parallel to the second, recalling the condition observed in Teleosts. Such variations in the Frog's egg have been figured by Prévost and Dumas ('24), Max Schultze ('63), Rauber ('83), and others.

Fifth cleavage. — In this cleavage (Fig. 9) the furrows are both horizontal and vertical, the majority being horizontal. If we follow this cleavage in detail (Fig. 16), we find the first fur-

row appearing in quadrant *d*, passing from the first to the third vertical in a horizontal direction. The next furrow appeared in quadrant *a* 2 min. later, extended in the direction intermediate between horizontal and vertical, and terminated in the second vertical; 1 min. later a horizontal passed from the second to the third vertical in quadrant *c*. In a few moments horizontals appeared simultaneously in the adjoining cells of quadrants *b* and *d*. A horizontal 1 min. later passes between the third and first verticals in quadrant *a*; 1 min. later verticals depart from the first vertical in quadrants *b* and *c*.

Although I have never found this cleavage entirely vertical, it has been observed in the Frog by Prévost and Dumas ('24). They say: "Au même instant deux nouveaux sillons parallèles à celui qui s'était montré le second sur la partie brune, viennent se dessiner sur elle d'abord sous la forme d'une trace légère, et bientôt ils atteignent une profondeur analogue à celle de leurs prédécesseurs. Cet hémisphère se trouve alors divisé en seize parties égales ou à peu près."

Later cleavage.—The following cleavage (Fig. 10) is likewise made up of both verticals and horizontals. The majority are generally vertical, occasionally horizontal. The corresponding diagram (Fig. 17) illustrates the times of the divisions. The interval from the beginning of the first to the last furrow is 15 min. A vertical section through the lighter and darker regions of such an egg is shown in Fig. 20. The cells forming the roof of the segmentation cavity (*sc*) vary in size and pigmentation, the larger being more deeply pigmented.

In the next cleavage (Fig. 11), as in the preceding, the furrows pass in both horizontal and vertical planes. In the corresponding diagram (Fig. 18) the times are but partially recorded; it being at this time extremely difficult to observe the cleavage over the entire surface. The interval between the first and last furrows is 20 min.

The differences in color observed in earlier stages have gradually become more pronounced, so that at present in nearly all eggs differences exist. The lighter portion fades out gradually in the region of the equator, while the darker portion has a more distinct line of demarcation. In many eggs the surface

views indicate an acceleration of cleavage in the least pigmented portion, yet sections (Fig. 21) show the region possessing most pigment to be that in which cell activity is greatest.

After the cleavage shown in Fig. 12 it is impossible to follow the divisions, even in areas ; but by magnifying the egg some 30 diameters individual cells may be observed. At this time the cleavage of any particular cell occurs at intervals varying from 1 hr. to 1 hr. and 45 min.

Fig. 22 represents a vertical section through the more and less deeply pigmented portions. In the more deeply pigmented portion the roof of the segmentation cavity is several layers of cells thick, while in the less deeply pigmented portion it has remained a single layer. The relation which the more deeply pigmented portion bears to the blastopore and to the future embryo will be discussed presently.

Experiments on cleavage.—Hertwig's ('93) recent experiments on the Frog and Triton, in which the second furrow was made to pass in a horizontal direction, and in which the path of the succeeding furrows bore a definite relation to the direction and degree of compression exerted, indicate that the planes of cleavage may be entirely changed through the effects of mechanical pressure.

Experiments of the same kind were made on the eggs of *Amblystoma tigrinum* in April, 1893, in the Morphological Laboratory of the University of Michigan. Unsegmented eggs, from which the envelopes had been removed, were placed between the cut edges of two glass slides and laterally compressed to one-half their equatorial diameter. In order to observe both poles of the egg, the experiments were carried on in glass dishes placed upon a mirror.

The first vertical in the 34 eggs examined showed no constant relation to the compressed surfaces, in 7 passing through the longest equatorial diameter ; in 9, through the shortest ; and in 18, between the two. The second verticals showed no greater variation than under normal conditions. The following cleavage was generally equatorial, yet in some cases, 3 out of 34, these furrows were vertical. Beyond this cleavage many

irregularities were observed, no greater, however, than occur in normal cleavage.

Some of the compressed eggs died before the neural folds were formed. A number, however, produced normal embryos, and the longitudinal axis, when compared with the drawings made of the first and second cleavage furrows, showed in the 15 eggs examined variations ranging from 5 degrees to 45 degrees.

As a result of pricking the egg, a minute quantity of protoplasm was extruded forming extra-ovates which remained attached to the surface of the egg until the embryos were well developed. In a number of cases eggs in the two-cell stage were pricked on either side of the first furrow. I more frequently found the extra-ovates on the same side of the median line of the embryo, but occasionally on opposite sides.

The extra-ovates when detached exhibit interesting phenomena, segmenting for a time synchronously with the egg; the cells arrange themselves so that lighter and darker hemispheres are formed. In short, the extra-ovate is a miniature egg. I have not been able to rear any of them beyond the blastula.

In 5 other experiments a delicate silk thread was tied around the egg in the first and second cleavage furrows. The results were likewise variable; in 3 eggs the long axis of the embryo formed right angles with the thread, in 2 oblique angles were formed.

While these experiments indicate that no constant relation exists between the axes of the embryo and cleavage furrows, they are open to the objection that abnormally compressed eggs may reveal nothing of the normal relations.

Rotation of ovum. — The continuous rotatory movement of the vertebrate ovum so often observed by the older naturalists; Leeuwenhoek, Carus, Sharpey, Bischoff, and others, is a phenomenon as yet unexplained. Sharpey, Rusconi, and Bischoff held this to be due to the presence of cilia, yet Barry, Aubert ('54), and others failed to detect their presence, the latter attributing the movement to osmotic action.

In Amphibia (Triton, Amblystoma, Rana) the rotation of the egg within the membranes is most noticeable during the

period between the late blastula and the closure of the neural folds. I have often observed this movement going on in a large number of eggs at the same time, some rotating in one direction, others in the opposite.

Clarke states that in *Amblystoma punctatum* the surface of the body is covered with cilia, at the time the neural folds close, by means of which it keeps up its rotary motion. I have endeavored to detect cilia by teasing in normal saline solution, also by osmic acid fixation, but without success.

4. CLEAVAGE OF PETROMYZON.

In the study of this form I have been greatly assisted through the kindness of Dr. Johnson, who has not only placed his material at my disposal, but also has allowed me to use some of his notes and drawings on cleavage.

First cleavage. — The first furrow (Pl. XIX, Fig. 1) appears at the superior pole in from 4 to 5 hours after fertilization, the ends may progress at a uniform rate or one may exceed the other in rapidity. The furrow is completed 15 to 30 min. later. In the majority of cases the cells are nearly equal, but exceptions occur; out of 57 eggs examined from different lots of material 12 showed quite decided variations. One of these variations is shown in Fig. 5.

So far as I am aware, the only observation showing a marked difference in the formation of the first furrow is that of Calberla ('78), who found the first cleavage in a horizontal plane, dividing the egg into two unequal parts. As this condition has never since been observed, it is fair to suppose that it occurs but rarely.

Second cleavage. — The formation of the second furrow begins in about 1 hr. 30 min. after the appearance of the first, progresses in a manner quite similar, and forms with it right angles. In most eggs a cross furrow is present; it may arise as in *Amblystoma* by the two furrows starting from widely separated points, or may be the result of a shifting of the blastomeres as described in *Amphioxus* by E. B. Wilson.

One furrow of the second set may extend at right angles to the first, while the other forms an acute or obtuse angle, or both may form acute and obtuse angles as shown in Figs. 10 and 11. Both furrows of the second set may fall far to one side of the upper pole, forming two larger and two smaller cells (Fig. 9). One of the furrows may reach the opposite pole before the other has started, or it may not appear at all as a vertical but as an equatorial; such a case was observed in the living eggs shown in Figs. 5 and 6.

Third cleavage. — The third cleavage occurs in about 1 hr. and 40 min. after the second. This set is often horizontal, occasionally vertical, again a part vertical, a part horizontal, and others which fall between the two so that it becomes impossible to select a type. A few of the more frequent variations are recorded.

A variation is shown in Fig. 10, where three blastomeres are cut off by horizontal furrows, while in the remaining quadrant no furrow is yet formed, although one of the fourth set (4) has appeared.

In Fig. 7, horizontals (3) are present in two quadrants only, giving rise to two smaller micromeres; while a third furrow (3) passes vertically, taking a direction and position which would permit its interpretation as one of the second set. Again, in the egg shown in Figs. 3 and 4, three quadrants are divided by verticals, while in the fourth a "tangential" (Kupffer, '90) is formed. In Figs. 12 and 13 we observe a set of verticals, and an entire absence of horizontals.

The variations recorded may offer a solution to what has hitherto given rise to no little perplexity concerning this cleavage. The majority of observers maintaining that this cleavage is horizontal while McClure ('93) holds the typical method to be the formation of a set of verticals followed by horizontals.

The succeeding cleavage is represented in Figs. 14 and 15. The directions of the furrows are so varied that their classification is impossible, as is evident not only from my own observation but also from the fact that scarcely two observers agree as to its nature. Max Schultze ('56) held that two more equatorials follow the third. Shipley ('88) states that it is followed

by two meridionals. According to Kupffer ('90) either may occur and from the third cleavage on no regularity exists. Kupffer also adds a third order which is called tangential. Comparing the variations in the cleavage of *Amphibia* and *Petromyzon* we observe in the second and third acts of the latter the irregularities manifested by the third and fourth of the former.

5. CLEAVAGE OF *COREGONUS*.

The observations were made largely on preserved material, yet notes made three years ago on the cleavage of the living egg have served as a guide.

First cleavage. — The first furrow may divide the blastodisc into two equal parts as in Pl. XIX, Fig. 16, where they lie at first closely together, but soon assume the condition indicated in Fig. 17, in which they lie quite apart; or they may later become widely separated at either end as in Fig. 18. In case the first furrow gives rise to two unequal blastomeres, which is the usual occurrence, it is impossible to classify the variations, since they range from almost equal size to a condition in which one is two or three times the size of the other, as in Fig. 19.

This inequality in size of the first two blastomeres is one often observed in Teleosts. Rauber ('83) observed it in *Gobius*, Agassiz and Whitman ('84) in *Ctenolabrus*, Henneguy ('88) in *Salmo*, and H. V. Wilson ('91) in *Gadus*, yet all produced normal embryos.

Second cleavage. — The second set of furrows may form right angles with the first, giving rise to four equal blastomeres (Fig. 22). They may pass from the same point at one side of the pole, resulting in two smaller and two larger blastomeres (Fig. 24). Again the two furrows may depart from points widely separated (Fig. 23) as in *Amblystoma* and *Petromyzon*. If two unequal blastomeres are produced by the first cleavage, a variation often occurs in which the smaller blastomere remains undivided (Figs. 20 and 21) during this cleavage. Again the larger may divide twice while the smaller undergoes but a single division, as in Fig. 25, or the opposite may occur (Fig. 26), although seldom observed.

In the eggs of *Merlucius*, studied by Kingsley and Conn ('83), "the blastomeres varied widely in size, and the segmentation furrows progressed at varying rates in different portions of the germinal areas." Agassiz and Whitman ('84), Henneguy ('88), and others have observed like differences in the size of the blastomeres of the second cleavage. I have never found the second furrows passing in a horizontal plane, yet Kupffer ('68) found this to be the case in the Herring, and List ('87) observed the same in *Crenilabrus*.

The condition represented in Fig. 27, presumably derived from a form like that shown in Fig. 23, is of rare occurrence.

The form depicted in Fig. 28 is one likewise seldom observed, and it may be said that in the White-fish the typical six-cell stage of other Teleosts is only one of the many variations.

The meridional nature of the third cleavage in Teleosts is generally agreed upon, with the exception of Brook ('86) who holds that it is equatorial.

In the eight-cell stage, if such it may be considered, the cells show endless variations in arrangement, the bilaterally symmetrical form shown in Fig. 33 being very seldom found, that indicated in Fig. 32 more often, and most frequently some variation of that observed in Fig. 30. A radial arrangement about a central cell (Fig. 29) is occasionally observed.

While the majority of investigators have considered the cleavage of the Teleost-ovum as fairly regular, we find the cleavage of *Coregonus* quite the opposite; we may also infer from the work of Rauber ('83) that in certain forms if any two blastoderms, in late cleavage, were superimposed the furrows would nowhere coincide. Ryder also states "that it is probably true that most eggs will be found to vary more or less notably."

The forms shown in Figs. 30, 31, and 32 are probably the derivatives of an unequal two-cell stage. The wide separation of these groups of cells is a point of interest. A like condition has been figured by Miss Clapp ('91) in the eight-cell stage of the Toad-fish, but nothing is said concerning its significance.

There often exists the peculiar condition indicated in Fig. 35 in which there is a marked separation of the derivatives of the early blastomeres. This at once suggests the beginnings of double embryos; while this seems very plausible, it is nevertheless improbable, since double embryos are but rarely observed.

Brauer ('94) has observed the same separation in the egg of the Scorpion, yet has not been able to determine whether the parts later unite or remain separated and form double embryos.

Another curious fact is the existence of extra-ovates which keep pace with the segmentation of the blastodisc and show no connection with it whatever. They appear to originate from the layer of cells extending over the yolk, which was presumably in an earlier phylogenetic stage covered by the blastodisc.

6. GENERAL CONSIDERATIONS.

Polarity.—One of the most striking features of the Amphibian ovum is its tendency to orient in such a manner that the darker hemisphere is uppermost. Von Baer in 1834 called attention to this difference between the upper and lower hemispheres of the egg, and by it determined the primary axis of the ovum. The distribution of pigment, the formation of polar globules, the initiation of cleavage, and the accompanying "Faltenkränzen" serve further to emphasize this polar differentiation.

Among those who first called attention to this phenomenon may be mentioned Auerbach, Balfour, and Lankester.

Hatschek ('77) states: "Es ist wahrscheinlich, dass eine polare Differenzirung schon an der ungefurchten Eizelle bei allen Metazoen statt hat, durch welche der vegetative und animale Keimpol bestimmt ist."

Whitman ('78) observed a marked polarity in the egg of Clepsine, and emphasized its importance.

Hallez ('85) has noted its occurrence in insect ova, and says: "La cellule-œuf possède la même orientation que l'organisme maternel qui l'a produite; elle a un pôle céphalique et un pôle caudal, un côté droit et un côté gauche, une face dorsale et une face ventrale."

Van Beneden ('83) observed this to be true not only in the unsegmented but also in the unfecundated egg of *Ascaris*. "Un examen attentif de la forme extérieure de l'œuf, et plus encore l'étude de sa structure, démontrent l'existence dans ces œufs d'un axe morphologique, dont les extrémités, que j'appelle les pôles de l'œuf, présentent une valeur tout différente tant au point de vue anatomique qu'au point de vue physiologique. L'un de ces pôles est prédestiné à recevoir le zoösperme : par ce point seul l'élément fécondateur peut pénétrer dans le vitellus."

One is accordingly forced to believe that we must assume a polarity not only in the unsegmented egg, but at a much earlier period, even preceding fecundation.

Mark ('90) has shown that in *Lepidosteus* the ovarian egg early exhibits a polar differentiation, indicated by a secretory activity of the protoplasm at a point which corresponds to the future micropyle.

In this connection the observations of Stauffacher ('93) on the eggs of *Cyclas* are of interest. There is a certain area, over which the egg membrane does not extend, which later becomes the micropyle. Stauffacher found this area to be the point by which the early egg-cell is attached to the follicular wall; one pole of the egg is thus pre-determined in the germinal epithelium.

One of the most noticeable features in the central nervous system of the larva of *Amblystoma* is the polarity of the nuclei indicated by the peculiar eccentric position of the chromatin. Mall ('93) likewise noted its presence in the cells of the retina of *Amblystoma*, and considered its importance in the development of the nerve fibre.

Rabl ('89) has called attention to a polarity existing in the nuclei of the epithelial cells of the Salamander.

In the nuclei of the intestinal gland cells of *Ptycoptera*, Van Gehuchten ('89) has observed this polarity and emphasized its importance.

The above facts not only indicate that the ovic axis is established at a very early period, but also that this is one of the most fundamental characters of animal cells; and we may con-

clude with Hallez ('86) that "chaque élément histologique possède, lui aussi, ces deux polarités de l'animal, polarités qui persisteraient dans la cellule-œuf, après qu'elle a cessé de faire partie des tissus maternels."

Relation of ovic axis and embryo to cleavage planes.—One of the first questions to be considered is the relation of the first furrow to the ovic axis, the upper pole of which is marked by the point of exit of the polar bodies.

Schultze ('87) holds that the plane of the first furrow is determined by the ovic axis and the eccentric germinal vesicle, and that its direction may be anticipated in the unsegmented egg.

Roux ('88), on the other hand, maintains that the first cleavage is determined by the ovic axis and the path of the male pronucleus, as first stated by Newport in 1853.

In *Amblystoma*, *Petromyzon*, and *Coregonus* this furrow does not always pass through the ovic axis, nor does it bear a constant relation to it, except that it generally passes in a plane parallel.

This peculiarity Rauber ('83) has noted, and suggests that there must be a "Polflucht" through the influence of which the furrows are thrown away. Whether this be accepted or not the fact remains, and is of great importance, that this eccentricity commonly occurs, hence the ovic axis cannot be considered as a rigidly determining factor.

Concerning the relation of the succeeding furrows to the first, it seems conclusively shown from the variations recorded in the preceding pages, as well as the observations of Rauber ('83) and Jordan and myself ('94), that in the forms studied no fixed relation exists.

Another theoretically important question is the relation of cleavage furrows to the axes of the embryo.

Newport, in 1853, called attention to this in the following words: "I have long been aware that the axis of the embryo was in line of the first cleft of the yolk." In a following paragraph, however, the author states: "The observations point out that at times the axis deviates to the right or left of the line."

From a number of experiments Roux, in 1883, reached the following conclusion: "Mit der Ebene der ersten Furchung

wird beim Froschei zugleich auch die künftige Medianebene des Individuums bestimmt, und zwar fallen beide zusammen." From the facts recorded by Roux (pp. 14-16) it is evident that this coincidence was by no means constant.

In the same year Pflüger ('83) reached the conclusion that the first furrow represented the median line of the embryo. Supported by these eminent investigators, the idea received very wide acceptance. Rauber ('86) was one of the first to question the observations, and showed that in the Frog and Axolotl this was not true, but that the second meridional instead represented the median plane of the embryo. By consulting Rauber's tables it will be observed, however, that the embryo was formed at almost any angle to the first cleavage. The later experiments of Hertwig on the egg of Triton, in which a silk thread was tied through the first furrow, confirmed the observations of Rauber, as do also the observations of Jordan on the Newt.

The work of Miss Clapp on so favorable an egg as that of *Batrachus* seems to show that in this form, at least, the first plane of cleavage bears no definite relation to the median plane of the future embryo.

In 1892 Roux modified the position which he held in 1883, and states that "Bei den bilateralen-symmetrischen Tieren entspricht eine der beiden ersten Teilungsebenen der Medianebene des Embryo resp. des erwachsenen Tieres."

In case the second furrow coincides with the median plane, it is interpreted as being intrinsically the first. Roux says: "Es entsteht dann die normalerweise zweite, kopf- und schwanzwärts scheidende Furche als erste, und die normale erste, der Medianebene entsprechende Teilungsebene als zweite."

To my mind a serious objection to all theories postulating the coincidence of any cleavage furrow with the median plane of the embryo in *Rana*, *Bufo*, *Amblystoma*, and *Diemyctylus* is the fact, emphasized by Jordan and myself ('94), that the furrows undergo a decided torsion. A glance at Pl. XVIII, Figs. 13-18, will illustrate the point. This shifting of the cells, although often observed, has hitherto received but little attention. It was witnessed by von Baer in 1834, and described

as follows: "Es ist ein wunderbares Schauspiel, unter der Loupe diesen plötzlichen Tumult in Dotter Klümpchen zu sehen. Manches Individuum wird von seinen unruhigen Nachbarn einmal hin und her geschoben bevor es zu Ruhe kommt."

Newport ('51) observed the same, and says: "In some ova there is such an unusual displacement of the segments as almost to prevent the identification of the parts in the subsequent changes." It thus becomes impossible for any furrow to coincide with a straight line forming the median plane of the embryo, unless after the egg has reached a very late stage, the cells shift back and arrange themselves along definite lines, which seems extremely improbable.

I believe the only rational conclusion which can be drawn is that no cleavage furrow in these vertebrates bears a fixed relation to the future median plane of the embryo.

Homology of cleavage furrows.—The supposed homology of the first cleavage furrows throughout the vertebrata is based upon its relation either to the ovic axis or the embryo. Since it bears no constant relation to either, it seems questionable if any homology can be drawn.

In attempting to homologize the second furrow more serious difficulties are encountered, since we must consider this cleavage as made up of two entirely independent cell divisions, the plane of which bears no fixed relations to the first furrow, either in time or direction, or to the ovic axis. If we compare the second meridionals in the two eggs of *Petromyzon* (Pl. XIX, Figs. 6 and 8), the difficulties are evident.

In comparing the Teleostean and Amphibian types of cleavage, Rauber was forced to postulate the loss of the first set of equatorials in the Teleost. H. V. Wilson believes such a loss probable and holds with Rauber that the equatorials in the Teleost are represented by the edge of the blastodisc.

Agassiz and Whitman think this improbable and accept the homology of the third cleavage in the two groups (according to Rauber and Wilson the third Amphibian and the fourth Teleostean).

It is evident that I cannot accept either of the above homologies and do not believe that even in the Amphibia we can

speak of the third cleavages as homologous. The difficulty lies in the fact that this so-called furrow is made up of four distinct furrows. As already shown, one, two, three, or four of these may pass in equatorial or in vertical planes and we are obliged to say, if we wish to homologize, that three-fourths of this cleavage in one egg is homologous with one-fourth in another, etc.

It is, of course, true that occasionally variations in the Teleost and the Frog correspond very closely, but to suppose that certain variations in the Teleost which happen to correspond to variations in the Amphibian are atavistic, as held by some, seems to me extremely hypothetical. The variations in *Petromyzon* and *Coregonus* are so frequent that one may say without exaggeration, that very rarely, if ever, at this stage the cleavages in two eggs of the same lot bear precisely the same relation to the ovic axis or the preceding furrows, to say nothing of the disparities in time and point of origin, direction, and rate of progress.

Jordan and myself repeatedly followed the cleavage of various Amphibia with the hope of discovering a law, but all attempts in this direction proved fruitless. It is even doubtful, as Mauro Rusconi stated in 1836, if the geometrical progression 2, 4, 8, 16, 32, 64, etc., formulated by v. Baer, ever occurs.

In the egg of *Amblystoma* the alternating periods of repose and activity are well marked in the early cleavage stages, but as development proceeds this synchronism gradually disappears. The differences of time existing in the periods of activity as indicated by the origins of the furrows may be tabulated as follows:

1st Cleavage, interval between appearance of first and last furrow	ca.	—	min.
2d " " " " " " " "	"	"	1-2 "
3d " " " " " " " "	"	"	4 "
4th " " " " " " " "	"	"	4 "
5th " " " " " " " "	"	"	5 "
6th " " " " " " " "	"	"	15 "
7th " " " " " " " "	"	"	20 "
8th " " " " " " " "	"	"	27-35 "
9th " " " " " " " "	"	"	40-50 "

The gradual obliteration of the distinct periods of rest, and the consequent transition to a condition of constant activity is brought about at an early stage. It is of interest when compared with the regularity manifested in the cleavage of certain Teleosts (Serranus, Wilson, '91).

The transition is due to the early appearance of areas of special activity. While the region of greatest activity is undoubtedly at the superior pole, there is another portion of the blastoderm in which cell division becomes accelerated; this is that portion which is most deeply pigmented and which is destined to lie somewhere in the future tail region of the embryo.

Kölliker ('79) observed these differences in the embryonic area of the chick. I quote his words: "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der andern voran ist . . . der schneller sich furchende Theil zum späteren hinteren Theil des Blastoderma sich gestaltet, in dem die ersten Spuren des Embryo entstehen."

In the blastoderm of *Amphioxus* Lwoff ('92) describes an area of accelerated cleavage at the dorsal lip of the blastopore: "die Zellenvermehrung nicht überall gleichmässig vor sich geht, sondern sich vorzugsweise an einer Seite konzentriert, die zur Dorsalseite der Gastrula wird."

The fact that areas of accelerated activity appear in early cleavage stages is, as we shall see, one of fundamental importance in orienting the embryo.

If we compare the variations observed in the three types studied, it is evident that the greatest variation is in the Teleost while the least is in *Amblystoma* and *Rana*. The order of variation might be expressed thus: the first and second furrows in *Coregonus* show the irregularities found in the second and third of *Petromyzon*, while the second and third of *Petromyzon* show about the same degree of variation as the third and fourth of *Amblystoma*.

From the preceding pages we conclude that in *Petromyzon*, *Amphibia*, and *Teleost* the more detailed the study of cleavage the more apparent become the irregularities, and we wonder

that from eggs manifesting such diversities of cleavage identical embryos, so far as we are able to determine, arise. If abnormal embryos resulted, the variations from a given type might bear a direct causal relation; but from the evidence bearing upon this point such does not appear to be the case, and my study only emphasizes the truth of the statement made by Jordan and myself ('91) that "the irregularities of cleavage have no appreciable effect upon any stage of development of the embryo."

The above being true, two alternatives present themselves. Either the egg is isotropic, or its mosaic character is not revealed by its mode of cleavage. If the former be true, we are wholly unable to explain the many facts of precocious differentiation so obvious in the Annelids, Molluscs, and other invertebrates. To accept the latter is to admit the inadequacy of the mosaic theory as based upon cleavage phenomena.

II. GASTRULATION TO CLOSURE OF NEURAL FOLDS.

I. FORMATION AND CLOSURE OF THE BLASTOPORE IN AMBLYSTOMA.

About 60 hrs. after the first cleavage gastrulation begins. In order to follow the process in detail the following methods were employed: The egg, while still within its membranes, was placed upon a glass slide, and after allowing the membranes to dry just enough to hold the egg firmly *in situ*, the slide was inverted and placed over a moist chamber. This method together with the assistance of a bed of cotton upon which the eggs, with envelopes removed, could be easily held in position, have enabled me to observe the movement of individual cells.

At the close of cleavage there is an irregular broken line (*bp.*, Pl. XX, Fig. 1) lying just below the equator and formed by the union of a number of cleavage furrows; along this line the process first begins. While in *Amblystoma* invagination always begins nearer the equator than the vegetative pole, other conditions have been observed in *Amphibia*. In *Rana* Pflüger ('83) and Roux ('88a) find the blastopore first appearing at the equator of the egg, while Houssay ('90) in the *Axolotl*,

and Jordan ('93) in the Newt, state that it first appears at the vegetative pole.

I have examined a large number of eggs at this stage to determine what relation this line bears to the more deeply pigmented area of the superior hemisphere to which I have already referred. In all eggs where the differences are sufficiently marked to admit of orientation, the irregular line lies beneath the darker portion and is parallel with its more sharply defined border. It will be recalled that the deeply pigmented area is the area in which cell-division has become much accelerated (Pl. XVIII, Figs. 21, 22).

Concerning this peculiar distribution of pigment there are but few references in the literature. Roux ('83) observed certain differences in the Frog as expressed in the following words: "Von der Gastrula ist es bekannt, dass schon bald nach Beginn ihrer Bildung die Rückenseite des Blastoporus an dunklerer Färbung kenntlich ist, womit zugleich der Punkt bezeichnet ist, an dem die Rückenfurche sich zu entwickeln beginnt."

Hertwig ('82) found in Triton "dass der Dotterpfropf keinen gleichmässigen Anblick darbietet, insofern eine Hälfte ganz pigmentfrei ist und weissgelb aussieht, die andere aber ein wenig bräunlich pigmentirt ist. Ferner ist auch der an die weissgelbe Hälfte des Dotterpfropfes angrenzende Theil des Eies viel schwärzer pigmentirt als die Umgebung der anderen Hälfte. Nach diesen Verschiedenheiten kann man sich über dorsal und ventral an der Kugeloberfläche orientieren, da die unpigmentirte Partie des Dotterpfropfes der Rückenfläche des Embryo zugekehrt ist."

Houssay ('90) observed the extension of the pigment to be "plus rapide sur le côté qui deviendra le dos de l'embryon."

While the point in question is not referred to, it is evident that the observations on the Frog, Triton, and Axolotl, as well as those of Kölliker ('79), on the Chick, and Lwoff ('92), on *Amphioxus*, accord perfectly with what I have found in *Amblystoma*. Morgan and Tsuda ('94) hold, however, that "in *Rana* the blastopore appears on the less pigmented and further developed side of the egg."

The stage shown (Pl. XX, Fig. 1) is soon followed by a sinking in of the cells on either side of the line, forming a depression which, in 8 hrs., becomes a crescentic groove (Fig. 2).

A corresponding stage of *Rana* is shown in Fig. 9. The blastopore (*bp.*) appears in the same region as in *Amblystoma*; very early the line of invagination (*bp.*) assumes the form of a more or less acute angle (Fig. 9), but to this angle but little significance can be attached beyond its slight bearing on the theory of concrescence.

As is well known, different opinions are held concerning the formation of the gastrula in *Amphibia*. A brief summary will define the views of a number of investigators.

The archenteron is formed by invagination, as held by Scott and Osborn ('79), Hertwig ('82), Schultze ('88), Perényi ('89), Jordan ('93), Morgan and Tsuda ('94).

These authors are, however, not agreed as to the precise method; the majority believe the process to be one of infolding (embolic invagination). Morgan and Tsuda consider it largely a process of overgrowth (epibolic invagination), while Jordan believes both processes are involved.

The archenteron is not formed by invagination, but by delamination, according to Moquin-Tandon ('76), Houssay ('90), Robinson and Assheton ('91), and Marshall ('93).

With the aid of the mechanical devices described, I have been able to follow certain cells, which from individual peculiarities could be easily distinguished, in their course from the point which they occupied at the rim of the blastopore, step by step, until they disappeared from view. It is evident, therefore, that there is either an infolding of the surfaces on either side of the line of invagination, or that the surface at the dorsal lip, is overgrowing the other, and at the same time infolding, giving such an appearance. Sections show that at this stage the ectoblast has overgrown the entoblast to a slight extent only, so that while there is some indication of epiboly, the appearance of sections and the actual infolding of cells observed on the surface can only be interpreted by considering the process as one of modified emboly.

The lateral extension of the groove soon reaches its limit, the ends turn toward the vegetative pole, and in 15 hrs. the crescentic blastopore has extended to the form shown in Pl. XX, Fig. 3. This is soon followed by the union of the ends completing the blastopore. At this time there is an infolding around the entire rim, the rate being most rapid at the dorsal margin, and gradually decreasing toward the ventral.

Fig. 7 represents a condition often observed where the blastodermic margin is defined at a much earlier period than in Fig. 3. A like condition was often found in *Rana* (Fig. 8) and in *Petromyzon* (Fig. 10).

The closure of the blastopore progresses gradually with the infolding. In 18 hrs. the condition shown in Fig. 4 is reached, when the external indications of gastrulation are beginning to fade. The blastopore has narrowed as a result of the unequal infolding. I am also inclined to believe that it is in part the result of mechanical pressure caused by the thickenings of the epiblast (*n.f.*) on either side of the dark hemisphere.

In a few hours the oval opening shown in Fig. 4 has been reduced to the slit-like aperture indicated in Fig. 5. This narrow slit is soon entirely closed at its center (Fig. 6), leaving an anterior opening lying just within the neural folds (*n.f.*), forming the neuropore (*np.*), and a posterior just without which persists and forms the anus (*a.*) as stated by Morgan ('80). This in *Amblystoma* is the usual method of closure, but it is by no means the only method. I have often observed a pear-shaped opening with the smaller end anterior, instead of the more usual dumb-bell outline. I have also followed the closure of the blastopore in *Rana palustris*, and am able to confirm the observations of Ziegler ('92), that the posterior portion of the blastopore persists as the permanent anus.

Hertwig ('92) states that in the Newt the "Urmundränder sich in einer von vorn nach hinten langsam fortschreitenden Richtung in der Medianebene zusammengelegt haben und verschmolzen sind."

Robinson and Assheton ('91) find in *Rana* that "the anus of *Rusconi* gradually diminishes in size by the condescence of the ventral parts of the lateral lips."

Marshall ('93) confirms the observations of Robinson and Assheton, stating that "the reduction is effected, not by a contraction of the whole circumference of the blastopore, but by a folding together, or concrescence, of its lips in the median plane, beginning at the lower or ventral margin and proceeding upwards towards the dorsal margin."

Such a procedure I have never observed in *Amblystoma* or *Rana palustris*, although Jordan ('93) found it occurring in the Newt.

Before the neural folds are well defined (Fig. 5) the neural groove (*n.g.*) appears; in some cases it runs forward as a continuation of the slit-like blastopore, while in others it is not continuous, but an independent groove (Fig. 5). A second groove is often observed lying between the posterior end of the neural groove and the blastopore; often it arises at, and is continuous with, the dorsal lip of the blastopore.

2. THE ORIGIN OF THE MESOBLAST AND CHORDA.

The study of the early mesoderm may best be introduced by examining a meridional section (Pl. XXI, Fig. 1) of such an egg as that shown in Pl. XX, Fig. 7.

The segmentation cavity (*s.c.*) in most cases shows a radial symmetry, although occasionally its bilaterality is strongly marked, the roof being composed of from two to three layers of cells. The slight infolding shown in surface views is here observed to occur along a line where the entoblastic cells shade off into the smaller epiblast cells. I shall henceforth speak of this infolded blastodermic rim (*g.r.*) as the germ-ring, since I believe it to be homologous with a like thickening in the blastoderm of Teleosts and Elasmobranchs to which this term is generally applied. Just within and above the line of invagination there is a group of cells (*m.*) which are larger than the adjoining ectoblastic cells, and possess more pigment than the entoblastic cells. These cells, extending around the entire egg, form a ring which later gives rise to the mesoblast.

Fig. 2 represents a vertical section along the line *xy* of Pl. XX, Fig. 2. The roof of the segmentation cavity is somewhat thinner than in the preceding stage, being in some por-

tions but a single layer of cells. The dorsal lip (*d.l.*) of the blastopore is well defined, the ventral being indicated by a slight thickening (*g.r.*) at the opposite side of the egg. At the dorsal lip in the slight angle formed by the infolded epiblast there are a few loosely scattered mesoblastic cells (*m.*).

Passing now to Fig. 3, which represents a vertical section along the line *xy* of Pl. XX, Fig. 3, we observe a decided differentiation of the mesoblast (*mes.*) even before the infolding is well marked. These mesoblastic cells, in size, pigmentation, and reaction to stains, more closely resemble the cells of the epiblast than those of the entoblast.

In sections (Fig. 4) through a still later stage (between Figs. 3 and 4, Pl. XX), the cleavage cavity is much smaller, having been gradually reduced as the gastral cavity has extended. The roof of the gastral cavity is made up of two kinds of cells, which merge along a fairly well defined region. The cells nearer the blastopore partake of the character of the epiblastic cells, while toward the segmentation cavity they resemble those of the entoblast. The thin anterior wall of the mesenteron, as well as the communication often found between the two, lead one to infer that a part of the segmentation cavity may often become continuous with that of the mesenteron.

In the angle formed by the invaginated hypoblast the mesoblast (*mes.*) is better defined. Some distance below the dorsal lip of the blastopore is a second group, which is the ventral portion of the ring (ventral mesoderm).

There is, then, at even this relatively late stage, a continuous layer of mesoblast around the blastopore. Soon a division occurs at the dorsal lip, due possibly to the pressure of the chorda hypoblast against the epiblast. Whatever may be the cause, the fact is, that before the blastopore is entirely closed there is no longer mesoblast in this region, while it is present and well defined on either side of the chorda.

The later growth of the mesoblast is through the antero-lateral extension of the wings lying on either side of the chorda and the postero-lateral extension of the continuous zone at the ventral lip of the blastopore.

While the majority of observers have denied the existence of a continuous layer of mesoblast at the dorsal lip of the blastopore, there are certain observations scattered through the literature which show that its presence in some forms, at least, is an undisputed fact. Götte ('75), in *Bombinator*, finds all three layers existing at the dorsal lip when the first indications of gastrulation appear.

Schultze ('88) says concerning the existence of the mesoblast in this region in *Rana*, that "Schon auf dem Stadium der Beginnenden Gastrulation in der dorsalen Urdarmrand drei Keimblätter existieren."

Houssay ('90) has found the same to be true in the *Axolotl*, and says: "L'étude de l'*Axolotl* confirme donc tout ce qui a été dit par Götte et Schultze, à propos des Anoures."

Perényi ('89) in *Bombinator* found the three layers of the epiblast to be folded in at the lip of the blastopore, the outermost becoming invaginated hypoblast, while the others gave rise to mesoblast.

Marshall ('93) states that in *Rana*, "At the lip of the blastopore, round its entire circumference, the three germinal layers, epiblast, mesoblast, and hypoblast, are indistinguishably fused together."

The chorda. — Concerning the origin of the *Chorda dorsalis* in *Amphibia*, no less than a score of investigators have recorded their opinions.

Among those who hold that the chorda is of entodermic origin are: Scott and Osborn ('79), Bambeke ('80), Hertwig ('82), Orr ('88), Houssay and Bataillon ('88), Robinson and Assheton ('91), and Jordan ('93).

Götte ('75) and Schultze ('88) dissent from the above, and maintain that it arises from the mesoderm.

When the infolding has progressed to the extent shown in Pl. XXI, Fig. 4, the outline of the chorda resembles the section of a cone. It consists of a single layer of invaginated hypoblast forming the roof of the gastral cavity (*g.c.*). As gastrulation proceeds, this layer of hypoblast extends and assumes an obovate form, consisting throughout of a single layer of columnar cells which on either side pass over into the

mesoblast. When the blastopore has narrowed to a slit, the chorda (*ch.*) consists of a single layer of hypoblast but few cells in width, as shown in Fig. 9.

Reconstructions show an irregular outline, the enlargements and constrictions often appearing in such order that they suggest the possibility of a metameric arrangement. The later development is essentially the same as observed in other Amphibia. The layer of columnar hypoblast folds up, and is gradually constricted until it lies free in the mid-dorsal region.

3. EXPERIMENTS.

Before considering the embryonic area, I wish to record the results of certain experiments.

The experiments were made on the eggs of *Amblystoma tigrinum* during March and April, 1893, in the Morphological Laboratory of the University of Michigan.

A method was sought by which I might be able to distinctly mark the surface of the egg and at the same time interfere as little as possible with normal development. After a number of methods were tried the following one was adopted: The outer envelopes are removed from the egg, which is then placed in a watch crystal on a bed of cotton, with barely enough water to cover it. The egg is then rotated until the desired point is uppermost, when it is punctured with an extremely fine hair held by forceps. A small quantity of the protoplasm oozes out and forms a minute extra-ovate, which remains attached to the egg. The eggs thus marked are transferred to watch crystals, in which they remain until the embryo is formed. A large mirror is fastened to the stage of a dissecting microscope; on this the watch crystals are arranged in series, and the eggs examined by means of an extension arm. The image of the opposite side may be thus observed during the entire period, from the time of marking until the embryo is formed. This is necessary, since the very small extra-ovates are easily detached.

Since to record these experiments in detail would require considerable space, I confine myself to a brief summary.

The center of the dark hemisphere was punctured in early and late segmentation stages, and the location of the extra-ovate observed when the embryo was formed. The results were variable. In 7 eggs the extra-ovate occupied a position near the median line, just beyond the region of the cephalic portion of the neural fold. In 5 cases it was located just within this fold; in 4, on the right side of the median line; and in 1, on the left.

The cause of these slight variations is probably due to the fact that punctures cannot be made at the same point in the different eggs.

From these experiments I conclude that the apical pole corresponds to the later head region of the embryo. This observation accords with what occurs in the Teleost egg, as stated by Professor Whitman in lectures on vertebrate embryology.

Schultze ('87) believes, however, that in *Rana* the apical pole represents the mid-dorsal region of the embryo.

Roux ('88^a), in the same form, holds that the apical pole forms the ventral side of the embryo.

Were it true that the apical pole later forms the ventral part of the embryo, we should expect to find considerable pigment in this region, as well as an area of smaller cells, which is not the case.

If at the first appearance of invagination a puncture is made in the dorsal lip of the blastopore, much variation occurs in the later position of the extra-ovate, probably owing to the difficulty in locating the median portion of the embryonic area. It will be recalled that one side of the groove may extend more rapidly than the other. If, however, the puncture is made at a time when the blastopore is crescentic or horse-shoe shaped, there is less difficulty in orienting the embryo. In all cases (17 eggs) the extra-ovate lies in the posterior third of the embryo.

Concerning the fate of the dorsal lip of the blastopore, we have but the observations of Schultze ('87) and Roux ('88^a). The former finds a defect in this region to later lie in the posterior portion of the embryo, while Roux finds that an injury produced here later lies near the transverse fold of the head.

If, when the blastopore has become horse-shoe shaped, punctures are made on the outside at either extremity, the extra-ovates generally (7 eggs) lie at the side of the slit-like blastopore, or are carried in (2 eggs). In 9 eggs markings at the ventral lip of the blastopore, when it had become circular, were found at the posterior end of the embryo.

The observations of Morgan and Tsuda ('94) show that the same is true in *Rana*; and further, if the puncture is made at the center of the vegetative pole, it later lies in the tail region of the embryo. Often it happens that the extra-ovate is infolded or overgrown.

In 13 cases, when the blastopore had become crescentic, punctures were made at the equator, on the opposite side of the egg. The extra-ovates later occupied a position on the ventral side of the egg, beyond the caudal end of the embryo. Roux ('88^a) states that a defect here is later found at the caudal end of the embryo.

4. THE EMBRYONIC AREA.

The older idea of von Baer was that the Frog's egg, like that of the other vertebrates, could be considered as made up of two parts, the germinal and nutritive, represented by the dark and light hemispheres respectively.

Pflüger ('83) holds that the embryo develops largely in the white hemisphere of the egg, but thinks it probable that the anterior portion of the medullary plate arises from the black hemisphere.

Schultze ('88), by following the path of certain local defects found on the surface of the egg, concludes that the embryo is formed entirely in the dark hemisphere.

Roux ('88) concludes from the fate of a number of artificial markings produced by a hot needle that the embryo develops almost exclusively from the inferior hemisphere. Hertwig ('92), from a study of abnormal forms, reaches practically the same conclusion.

Morgan and Tsuda ('94) state that they in the main point agree with Pflüger and Roux.

The results of my observations are : First, an area at the apical pole forms the basis of the head of the embryo.

Second, the area at the dorsal lip of the blastopore lies in the posterior half of the embryo.

Consequently, the anterior half of the embryo, at least, is formed from the material lying between the apical pole and the dorsal lip of the blastopore.

The formation of the posterior portion of the embryo is so intricate that I hesitate to attempt a description, realizing that more careful study is necessary before anything like a complete explanation can be offered. We know that two processes, that of overgrowth and infolding, are going on during gastrulation; that these vary in rate in the different portions of the blastoporic rim, the most active area being at the dorsal lip. This variable rate of infolding, in connection with the pressure necessarily resulting from the thickened areas of the medullary folds, make the closure of the blastopore an extremely complicated process.

That this closure may occur in a number of different ways is undoubtedly true, as the observations of Schanz ('87), Morgan ('89), Erlanger ('91), Robinson and Assheton ('91), Ziegler ('92), Jordan ('93), and others, amply testify. I cannot consider this or that variation as indicative of the method of embryo-formation, but rather as secondary modifications of the more primitive process of uniform convergence.

The above facts indicate that the greater portion of the body of the embryo is formed in the dark hemisphere by differentiation *in situ*, while the tail arises from the coalescence of the blastoporic lips.

5. THE PRIMITIVE STREAK AND GROOVE.

Throughout the literature scarcely two statements can be found which agree as to the nature and extent of the primitive streak. In the Amphibia this is especially true, as the following brief references indicate :

1. The primitive streak is in front of the blastopore, Alice Johnson ('84), Schultze ('88), Erlanger ('90).
2. The primitive streak lies behind the blastopore, Minot ('92).

3. The primitive streak represents the part of the blastopore between the neurenteric canal and anus, Schwarz ('89).

4. The primitive streak represents the entire fused area around the rim of the blastopore, Robinson and Assheton ('91).

The term "primitive streak" as is well known, was first applied to an opaque streak in the posterior portion of the area pellucida of the chick; this streak, according to Balfour, is due to the presence of a third layer, the mesoblast. The later authors have quite generally agreed that the primitive streak is the portion of the blastoporic area where all three layers are fused.

This area of fusion, as stated in a preceding page, extends around the entire blastoporic margin, so that in *Amblystoma* the basis of the primitive streak is a well defined ring. It is not, however, until the closure of the blastopore that the structure is generally referred to as such.

I wish here to refer to a structure which has been interpreted as a more or less exact criterion of the extent of the primitive streak, *viz.*, the "dorsal groove." It would indeed be a hopeless task to attempt the enumeration of synonymous terms which have been given by the various authors, or to record the many views regarding its position and extension.

In *Rana temporaria*, according to Hertwig ('82), the dorsal groove extends forward from the blastopore, while in the same form Robinson and Assheton ('91) find it extending backward from the blastopore. In the Newt Hertwig ('82) never finds the groove continuous with the edge of the blastopore, while Miss Johnson ('84) often finds this occurring. Jordan ('93) observed the primitive streak and neural grooves to later unite and form one structure. In a recent publication Bambeke ('93) has endeavored to show that the dorsal groove (*Rückenrinne*, *sillon médian*) represents the primitive groove and is the outward expression of the extent of the primitive streak. The author says:

"On peut admettre avec O. Hertwig, que le sillon médian ou dorsal (*Rückenrinne*) des Amphibies représente la ligne de suture, suivant laquelle, en vertu de leur rapprochement lent d'avant en arrière, les lèvres du blastopore se sont juxtaposées

et fusionnées. Le sillon médian est donc comparable à la formation désignée par Hatschek sous le nom de raphé gastrulaire (Gastrularaphé) des Ascidies, de l'Amphioxus et des Annélides."

These different views manifestly rest upon different conceptions of the extent of the blastopore and its method of closure.

A second source of confusion lies in the fact that the primitive, neural, and dorsal grooves are considered as one structure. In *Amblystoma* and *Rana palustris* there is a primitive groove, but this groove is not in front or behind the blastopore. It is the blastopore, or its remnant. In section, the line representing the area along which the lips have fused, is always present beneath it. A second groove is also often present, extending forward from the dorsal lip of the blastopore. It may appear as a slight depression just in front of the dorsal lip, extending in either direction, uniting the primitive and neural grooves, or it may be entirely absent. Sections through this groove show no line of fusion, nor in any way suggest that such has occurred.

The one fact which I wish to emphasize, is that this groove is in no way indicative of the extent of the primitive streak, but is a part of the neural groove, having arisen in precisely the same manner, while the primitive groove has an entirely different origin.

6. THE EPIBLAST AND MESOBLAST IN PETROMYZON.

A glance at the literature on this form shows a wide disparity of views concerning the process of mesoblast formation and extension. This, together with certain facts concerning the thinning of the roof of the segmentation cavity, preceding gastrulation, have led me to record a part of my observations.

In a late cleavage stage the roof of the cavity is composed of from two to three layers of cells, resembling the condition already noticed in *Amblystoma*; just before gastrulation the roof thins to a single layer of cells (Pl. XXI, Fig. 5), as stated by Schultze ('56), Scott ('81), and Shipley ('88). Kupffer ('90) and Hatta ('92), have likewise observed the thinning, but hold that the single layer is not reached.

A certain amount of this thinning may be due to a migration of the cells toward the equator, as held by some authors, yet I am satisfied that this is not the full explanation, since if the cells passed around the sides they should be recognizable from their smaller size. Moreover, it is impossible to interpret such appearances as shown in Fig. 5, except on one of the following hypotheses: Either these cells are wedging themselves in between the cells of the external layer, or they are being pressed out from between them. The former seems the more probable, since one must, at this time, think of an extension of the superficial layer rather than a reduction. Besides these cells possess coarser granules than the cells of the external layer, which accords perfectly with the former view, but not the latter.

Regarding the formation and extension of the mesoblast I have found no two authors in complete agreement.

Calberla ('78) states that the mesoderm arises through the division of the primary entoderm into mesoderm and secondary entoderm.

According to Scott ('81) the mesoblast is derived from two sources: One portion arises from the invaginated blastoderm, and a second portion is derived from the outermost layer of yolk cells. The latter completes the mesoblast on the ventral side of the body.

Nuel ('81) holds that a layer of hypoblast, lying between the layer forming the roof of the alimentary canal and the epiblast, gives rise to the mesoblast.

Shipley ('88) describes the mesoblast as arising from two bands of yolk cells which lie in the angles formed by the mesenteron and the epiblast. The differentiation begins in front and is continued backward.

Kupffer ('90) agrees with Shipley that the mesoderm extends from before backward, and when the region of the teleoblast is reached its growth is supplemented by cells derived from this group.

Hatta ('92) believes the mesoderm to have a double origin. The part arising first is the "peristomial," which is found around the entire rim of the blastopore, save a point at the

dorsal lip. A second portion, forming the gastral mesoblast, arises on each side of the chorda.

Pl. XXI, Fig. 5, represents a vertical section through the area of deepest invagination in an egg, at the stage shown in Pl. XX, Fig. 10. Lying between the epiblast and the invaginated hypoblast is a group of indifferent cells (*m.*), which forms the basis of the mesoblast.

A later condition is represented in Fig. 6. The group of cells (*mes.*) designated (*m.*) in Fig. 5, are so highly differentiated that they plainly constitute a third layer. This layer extends around the entire periphery of the egg.

The majority of investigators have not recorded the existence of mesoblast at the dorsal lip of the blastopore, yet Scott ('81) has described it as follows: "In der dorsalen Blastoporuslippe vermehren sich die Mesodermzellen sehr rasch und bilden in der Mittellinie eine continuirliche Platte."

In Fig. 7, after Hatta, a later stage is shown. The segmentation cavity has disappeared, owing to the extension of the gastral cavity. At this time there is a group of cells at the ventral lip of the blastopore (*v.mes.*), which Hatta interprets as the ventral mesoderm, and further states that "mesoblast cells are budded out from all around the lip of the blastopore, except the dorsal median point, where the epiblast turns around to join the hypoblast."

I at once endorse this statement, but would add that in an earlier stage the mesoblast also exists at the dorsal lip; its division at this time is probably due to the pressure of the axial string of invaginated hypoblast. Thus, in *Petromyzon*, as well as in *Amblystoma*, there exists a well-defined layer of mesoblast around the entire blastopore.

In a still later stage (Fig. 8), there is a splitting off of certain cells from the layer forming the roof of the gastral cavity. These form a caudal knob (*tel.*) to which Kupffer ('90) has given the name "teleoblast." Kupffer expressly states that the cells take no part in the early formation of the mesoblast. "Ausgangspunkt der Bildung des Mesoderm ist also hier bei P. Plan. nicht der Teloblast, überhaupt nicht der Blastoporusrand." As will be observed from the figures, this stage is

much later than those in which the origin of the mesoderm has been described, and when there exists in the axial region the two sheets of so-called "gastral mesoderm."

7. GASTRULATION AND FATE OF THE GERM-RING IN AMIURUS AND LOPHIUS.

If the two eggs be compared just before gastrulation, we observe a decided difference in the relative amounts of blastodermic and yolk material. In *Amiurus* the mass of the blastodisc is to the mass of yolk as 1:8. In *Lophius* the relative proportions would be as 1:20. As the blastoderm extends there is a thinning of its central portion and a thickening and infolding of its margin. In a certain region the infolding is most pronounced, giving rise to a projecting tongue (Fig. 17) which first defines the long axis of the embryo. A point directly behind, at the edge of the thickening, will be henceforth referred to as the provisional hind-end of the embryo. We may also speak of the thickened blastodermic rim as the germ-ring.

Passing to later stages of *Lophius* (Fig. 18), we observe the first outlines of the embryo, terminating at its provisional hind-end in a caudal knob (*c.k.*). A dorsal groove, wanting in many Teleosts, is here present, extending from the anterior end of the embryo to a point just in front of the knob.

In *Amiurus*, Fig. 12, the head-end of the embryo is outlined; posteriorly, however, it is not yet defined. The dorsal groove is well marked and communicates with the blastopore. In this form the caudal mass is absent; there are, however, slight prominences on either side of the dorsal groove (Fig. 13) which may be its representatives. The discovery of this bifid condition of the posterior end of the embryo of *Amiurus* is due to Miss O'Grady, of Vassar College. It is of importance, as it is additional evidence of concrescence.

Later stages of *Lophius* (Figs. 19, 20) show the gradual extension of the germ-ring over the yolk and a corresponding differentiation and extension of the embryo; the dorsal groove has disappeared and the optic vesicles are outlined.

In *Amiurus*, Fig. 14, at a stage when relatively the same amount of yolk remains uncovered, we find the embryo less dif-

ferentiated, the dorsal groove is deep and wide, there is no indication of optic vesicles, and the embryo is obviously in a much earlier stage of development — a fact of no little theoretical interest as an illustration of the relation existing between embryo and yolk. If these forms be compared with *Coregonus* (Fig. 16), the effect is more pronounced, the blastopore being almost closed before the outlines of the embryo are distinct, recalling the condition observed in *Petromyzon* and *Amphibia*.

Fig. 21 represents a later stage of *Lophius*. The germ-ring shown in Fig. 20 constricts and is represented by the two portions surrounding the vesicles *c.v.* and *c.v.'* In some cases the anterior ring is united on either side with the provisional hind-end of the embryo. In others the tail apparently lies free, the ring joining the embryo at a point considerably anterior.

Fig. 13, Pl. XXI, represents a median saggital section through an embryo at the stage just described. The provisional hind-end is well defined; the real hind-end being now at *g.r.*, and consisting of a mass of cells easily distinguished from the surrounding periblast (*per.*). The anterior ring is continuous with the provisional hind-end and the area enclosed by it is filled by a layer of periblast (*per.*), forming the roof of the cavity (*c.v.*). The walls of this cavity are lined with periblast. Lying immediately posterior to the vesicle (*c.v.*) is another (*c.v.'*), likewise lined with periblast. The layer of periblast forming the lateral walls and roof of these vesicles is of considerable thickness, while the portion forming the floor is of such extreme tenuity that it is often impossible to trace it throughout its entire extent. The roof of the anterior vesicle (*c.v.*) is formed by a layer of periblast only; over the posterior there is usually an additional layer of epiblast (*ep.*). I believe this drawing over, or extension, of the epiblast to be a secondary condition which offers no serious objection to considering the posterior ring as representing a portion of the original germ-ring. I have not been able to observe its formation in the living embryo, consequently am unable to say more.

Beneath the body of the embryo, at its posterior end, lies the peculiar vesicle (*K.v.*) to which Kupffer first called attention.

A later stage is represented, Pl. XX, Fig. 22, in which the anterior vesicle is much reduced in size; a slight constriction marks the boundary between the two portions of the germ-ring. Sections of this stage (Pl. XXI, Fig. 14) show the blastopore (*bp.*) to be also reduced in size. The periblast (*per.*) forming the yolk-plug is thickened; so far as I am able to determine the caudal vesicle (*c.v.*) does not open to the exterior.

The section Fig. 15 represents a stage when the closure of the blastopore is nearly complete; the continued thickening of the periblast is obvious; within it are a number of minute caudal vesicles (*c.v.*), which may represent the remnant of the anterior cavity (*c.v.*). The large cavity (*c.v.'*) seems to have undergone no farther change during this interval. Its epithelial covering is on all sides continuous with the tail-end of the embryo. The size of Kupffer's vesicle has remained practically unchanged.

The embryo soon grows away from the constricted portion and the condition represented in Pl. XX, Fig. 23, results, when there is no longer communication as sections shown with the body of the embryo. Many variations exist in the distance between the posterior end of the embryo and the extra embryonic portion of the germ-ring depending upon the relative rate of growth of the embryo.

In an extremely late stage (Fig. 24), when the embryo has almost reached the larval condition, the ring is still present. Sections at this stage (Pl. XXI, Fig. 10) show that the size of the posterior vesicle is somewhat reduced. The periblastic ring, however, remains well defined. The section Fig. 11 is a more highly magnified picture of the region marked *per.* in Fig. 10, showing the aggregation of periblastic nuclei in this locality, also the continuous layer of epiblast (*ep.*). Since these are the latest stages in my possession, the fate of this part of the germ-ring remains undetermined.

The striking similarity of Kupffer's vesicle to the caudal vesicles *c.v.* and *c.v.'* and the close connection often observed, amounting in some cases (Fig. 14) to almost a complete fusion, lead one to infer that these vesicles have arisen from an originally single vesicle.

In the development of *Amiurus* a like phenomenon is observed, although less marked, owing to the existence of relatively less yolk. In the stage indicated in Pl. XX, Fig. 15, the embryo is well developed and in about the same stage as that of *Lophius* just described, having almost reached the larval condition. At the point where the tail of the embryo is attached to the yolk there is a streak (*g.r.*) extending ventrally to a point considerably removed as indicated in the figure. This is but faintly outlined in the unstained egg, but by surface staining it becomes strongly contrasted with the surrounding surface.

A section across this streak reveals the condition shown in Pl. XXI, Fig. 12. The periblastic layer is well defined and the nuclei are aggregated in groups in such a manner that it suggests a fusion of two portions. Lying above these two groups are two corresponding thickenings of embryonic tissue separated by a more or less distinct groove. In fact the entire appearance of the section at once leads one to suspect that it represents the fusion of two bands of embryonic tissue. I interpret it as due to precisely the same process which we have already noted in *Lophius* and which Miss Clapp has observed in *Batrachus*, namely: The embryo differentiates so rapidly that there is left behind a certain part of the germ-ring which never enters into its formation.

I believe this fact one of great importance in the conception of the formation of the embryo, and to which I shall revert presently.

8. THE MESOBLAST IN FISHES.

I have endeavored to determine the origin of the mesoderm in *Amiurus*, *Lophius*, and *Coregonus*, but as might be anticipated from the poorly differentiated condition of the cells of the different layers, these forms, like other Teleosts, are unfavorable for deciding critical points. While the majority of investigators first speak of the mesoblast when it is recognizable as two lateral bands, there are others who have considered it as primarily a continuous layer: Hoffmann ('81), Kowalewsky ('86), McIntosh and Prince ('90), p. 728.

Concerning the origin of the mesoblast in Ganoids the literature is meager. Salensky in describing its origin in *Accipenser* says:

“Pour étudier les premières phases de la formation du mésoderme, il faut remonter jusqu’à l’apparition du blastopore et de la cavité digestive primitive.

“Les cellules entodermiques adjacentes au bord du bourrelet marginal se divisent bien plus tôt que les cellules centrales et les cellules entodermiques avoisinant le pôle inférieur de l’œuf. Quand ces cellules entodermiques déjà réduites sont refoulées sous le bourrelet marginal, quelques-unes d’entre elles se maintiennent avec leurs caractères primitifs, sous les cellules entodermiques différenciées, pour former la paroi dorsale de la cavité digestive. Ce sont ces cellules entodermiques réduites, après s’être multipliées, qui constituent le premier rudiment du mésoderme.”

Balfour and Parker give no account of its origin in *Lepidosteus*.

I have observed appearances in *Amia* which lead me to believe that the mesoderm forms as a continuous layer around the margin of the blastopore.

In Elasmobranchs there are a number of scattered observations to which I may briefly refer.

In the development of *Torpedo*, Swaen ('87) finds that “Dans toute l’étendue de la portion extra-embryonnaire du blastoderm, le mésoblaste a pour origine cette zone cellulaire périphérique dans laquelle les éléments épi- et hypoblastiques sont mélangés et qui établit la continuité entre l’épiblaste et l’hypoblaste primitif.”

In the same form Schwarz ('89) found the mesoderm continuous around the entire blastoderm; to quote his words: “Hier ist, wie überhaupt im ganzen Randwulst, das Mesoderm seitlich mit dem Entoderm in kontinuierlichem Zusammenhang.”

Kastchenko ('88), from a study of *Pristiurus*, *Scyllium*, *Torpedo*, and *Raja*, concludes that the mesoblast has a paired origin, but that later a continuous ring is formed. “Die freien Enden des peripheren Mesoblastes umwachsen nach und nach die ganze Keimscheibe und verwachsen mit einander am vor-

deren Rande der letzteren, indem sie einen geschlossenen Ring bilden."

The Zieglers ('92), in addition to the gastral mesoderm, describe a layer extending around the entire periphery of the blastoderm: "In den nächsten Stadien schreitet die Bildung des peripheren Mesoblastes rings um das ganze Blastoderm herum fort."

The above observations all point unmistakably toward a primitively unpaired condition of the mesoblast in Elasmobranchs. The formation of the axial mesoderm in some cases preceding the differentiation of the anterior portion of the peristomial is precisely what might be anticipated, since we would expect to find the process much retarded in this region, owing to the fact that through the great increase of yolk substance the most active area is far removed from that portion of the germ-ring which must be considered as representing the tail-end of the embryo.

I have purposely omitted referring to the origin of the mesoderm in Amphioxus, since Lwoff and E. B. Wilson have denied the existence of the pole cells, which, according to Hatschek, form the basis of the mesoderm.

9. GENERAL CONSIDERATIONS.

The evidence that the basis of the mesoblast in vertebrates is a closed peristomial ring may be briefly summarized as follows: I have demonstrated this fact in *Petromyzon* and *Amblystoma*. Its existence in Elasmobranchs is beyond question. Its presence in Teleosts has been repeatedly recorded. It explains certain peculiarities observed in Sauropsida and Mammals which have hitherto been unexplained, *viz.*, the formation of a part of the mesoderm at the periphery of the blastoderm, as observed in *Tryonix* and *Clemmys* by Mitsikuri ('91), and in *Sorex* by Hubrecht ('90).

I accordingly think the hypothesis well founded that the vertebrate mesoblast must be considered as primarily peristomial. The axial portion (axial mesoderm) in Amphibia has been carried in by invagination; while in forms containing a great amount of yolk substance, and where overgrowth has replaced invagination, the axial position is due to conrescence.

Concrescence.—Following the observation of Kowalewsky ('71), who considered the dorsal groove in the Frog, Amphioxus, and Ascidians as representing a continuation of the gastrula invagination, the papers of His ('76) on the Teleost and Rauber on the Chick clearly formulated the Theory of Concrescence, the evidence of this process in Amphibia being based upon the statement of Kowalewsky.

The presence of this groove has been considered by a number of later writers as evidence of concrescence.

Since I have already dwelt upon this point (p. 376), I refer the reader to the criticism there offered.

According to Roux the Amphibian embryo is formed by concrescence in the following manner: "Das Material zur Bildung der Medullarplatte jederseits durch seitliches Herabwachsen vom Äquatorrande aus auf die Unterseite des Eies geschoben wird, und dass diese von beiden Seiten her entgegenwachsenden Platten unten in der Medianlinie mit einander verschmelzen. Diese Verschmelzung findet successive und zwar in cephalocaudale Richtung statt."

This conception is based largely upon the experimental determination of the fate of the cells at the dorsal lip of the blastopore, which region, according to Roux, represents the head of the embryo.¹ If this one observation becomes an established fact, it will afford very conclusive evidence of concrescence. It must at present, however, be considered as a tentative hypothesis, since the observations of Schultze ('88), as well as my own experiments are far from confirmatory.

From a study of pathological forms Hertwig ('92) likewise holds that the embryo is formed by concrescence. "In der Rückenlinie erblicke ich die Nahtlinie, in welcher bald nach dem Beginne der Gastrulation die Urmundränder sich in einer von vorn nach hinten langsam fortschreitenden Richtung in der Medianebene zusammengelegt haben und verschmolzen sind." I am unable to discover whether Hertwig maintains two altogether different processes in Triton and the Frog, or whether he retracts the belief which he previously stated ('82),

¹ The late experiments by Morgan (*Anat. Anz.* 1894, p. 698) confirm the observations of Roux.

viz., "Nach vorn vom Urmundspalt und in geringer Entfernung von ihm senkt sich die Oberfläche des Eies zu einer kleinen Furche ein, die mit der Längsaxe des Eies zusammenfällt. Sie soll als Rückenrinne bezeichnet werden. Mit dem gleichgerichteten Urmundspalt fließt sie weder Anfangs noch auch später zusammen, sondern bleibt von ihm durch einen queren Wall getrennt, wodurch deutlich bewiesen ist, dass beide Bildungen in ihrer Genese vollkommen unabhängig von einander sind." Farther, p. 307: "Wenn die Primitivrinne der Vögel, wie jetzt vielfach angenommen wird (Gasser, Rauber, Braun) als Verschlussstelle des Urmundes angesehen werden muss, so entspricht sie dem Blastoporus der Amphibia, welcher später ebenfalls zu einem kurzen Langsspalt auswächst, dann aber kann sie nicht mit der Rückenrinne der Tritonen verglichen werden. Denn die letztere bildet sich vor dem Blastoporus, in einer Gegend, wo derselbe niemals gelegen hat, und ist von Anfang an durch einen Wulst von ihm getrennt. Das ist der Grund, warum ich den Namen Primitivrinne nicht für sie gewählt habe."

If the figures of abnormal embryos given by Roux ('88^a), Hertwig ('92), Morgan and Tsuda ('94), are compared, we observe marked differences in the extent of the blastopore. According to those given by Roux, its anterior limit is at a point just within the transverse medullary fold. Hertwig's Figs. 8 and 15, Pl. XVI, would indicate that it must extend far beyond, even to the region occupied by the sucking discs. In Figs. 17, 18, and 19 by Morgan and Tsuda the blastopore cannot be interpreted as extending even to the posterior border of the transverse fold.

These differences, together with the very great individual variations, make the evidence from this source very unsatisfactory. For my own part, I question the entire evidence adduced from pathological forms.

Minot's ('92) principal reason for believing concrescence to occur in Amphibia is the assumption that the dorsal lip of the blastopore, or rim of the blastoderm, becomes farther and farther removed from the segmentation cavity. Were this the case, it would be no more evidence of concrescence than of

overgrowth. A comparison of successive stages during gastrulation shows a continual diminution of the segmentation cavity, due to the extension of the gastral cavity, the part farthest removed from the dorsal lip being the last to disappear.

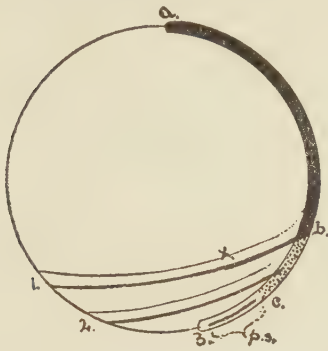


FIG. 1.

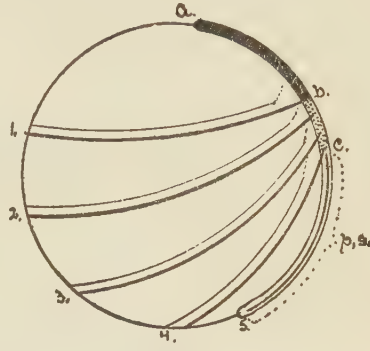


FIG. 2.

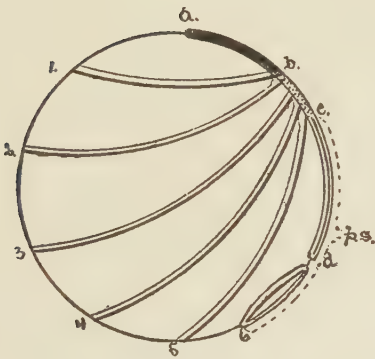


FIG. 3.

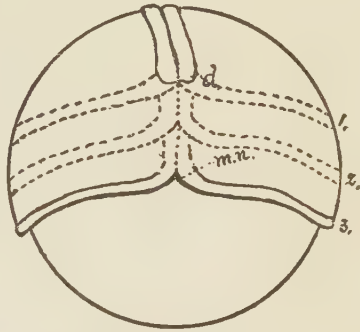


FIG. 4.

FIG. 1. — Diagram showing the formation of the embryo as observed in Amphibia.

FIG. 2. — Ditto in Coregonus and most Teleosts.

FIG. 3. — Ditto in certain Teleosts (*Batrachus*, *Amiurus*, *Lophius*) and Elasmobranchs.

FIG. 4. — Ditto in Chick after Rauber (modified).

The portion of the embryo (*a.b.*) formed by differentiation *in situ* is represented in black; that formed through backward extension, or overgrowth (*b.c.*) by dots, while the extent of concrescence forming the primitive streak is represented by the heavy line *p.s.*

The successive positions occupied by the germ-ring, or thickened blastodermic rim, are represented by 1, 2, 3, 4, etc.

These observations are the principal ones thus far brought forth to support the theory of concrescence in Amphibia, and to my mind they are far from convincing.

The accompanying diagrams are introduced to illustrate the modification arising in the formation of the embryo through a

constant increase in yolk, as compared with the mass of embryonic material.

In Fig. 1 I have attempted to represent the method of embryo formation in Amphibia, based on the study of *Amblystoma*. At a time when the blastopore extends around the entire blastodermic margin (1 *b*.) the first outlines of the embryo are barely visible; this portion of the embryo, extending to a point somewhere in the vicinity of *b*, is the part which is to be regarded as having formed through differentiation *in situ*.

Successive stages in the closure of the blastopore are represented at 1, 2, and 3. The closure in the earlier stages is effected through a convergence of the entire margin, which gradually changes to a lateral convergence, the margins finally coalescing to form the primitive streak (*p.s.*). From the position of the primitive streak one naturally infers that the ventral portion of the blastoderm must pass over the yolk most rapidly; the explanation probably lies in the fact that the effect of overgrowth is here least obscured by infolding. In order to comprehend the backward extension of the embryo from *b* to *c*, it is necessary to recall that at the dorsal lip of the blastopore there is an area in which the cell activity is greatest; otherwise the decided infolding at this point would appear incompatible with such an extension.

If we next consider a condition Fig. 2 (*Coregonus* and other Teleosts), we find the embryo has reached the same stage of differentiation as in Amphibia at a time when the blastoderm has extended over about one-third of the yolk. In other words, the embryo differentiates at a relatively earlier period than in Amphibia.

With the change in quantity and quality of yolk a corresponding modification has arisen in the process of gastrulation; there is no longer as extensive an infolding as in the preceding forms, while overgrowth must be considered as playing a more important part. We must also bear in mind the fact that the most active or formative area at *b* is farther removed from that portion of the germ-ring destined to form the tail of the embryo; so that during the time the germ-ring is passing over the yolk (1, 2, 3, 4) the embryo becomes well differentiated.

It is thus perfectly obvious that if this portion of the germ-ring ever enters into the formation of the embryo it must do so at a relatively later period than in Amphibia. The process by which the greater portion of the germ-ring is converted into embryo is an antero-posterior coalescence (concrecence) of its lateral portions, forming the primitive streak (*p.s.*). In accepting concrecence as the method, I adopt the view of His, that the head is practically a fixed point. In this diagram I have also represented the head of the embryo as arising at the apical pole. This is based directly upon the unpublished observations of Professor Whitman. The extent of the backward growth of the embryo through proliferation is difficult to determine. I have represented it as extending from *b* to *c*, rather to illustrate the fact that a certain portion is thus formed than to indicate its exact proportional extent.

Fig. 3 represents a still greater increment in the mass of yolk, as compared with the blastodisc (*Amiurus*, *Lophius*, *Batrachus*, and *Elasmobranchs*). The first outlines of the portion of the embryo (*ab*), which is to be considered largely the result of a differentiation *in situ*, appears at a time when the blastodisc covers a smaller portion of the yolk than in the forms represented in Fig. 2, its margin being indicated by the line *1b*. The successive positions occupied by the germ-ring and corresponding elongation of the embryo are indicated, as in the preceding, by the numerals 1, 2, 3, 4. When the region indicated by 5 is reached the ring constricts, leaving a part of the embryo (*6b*) behind. This is due to the fact that the formative area is so far removed from this portion that it cannot pass over the yolk before the embryo is formed.

This peculiarity, first observed in *Batrachus* by Miss Clapp ('91), and which I have observed in *Amiurus* and *Lophius*, illustrates the manner in which the "yolk blastopore" (Balfour) of *Elasmobranchs* has been formed, and offers a complete explanation of its morphological significance.

In a rare form of the Chick blastoderm, described by Whitman ('83), and which I have illustrated in Fig. 4, there was an extension of the primitive streak (*p.s.*) beyond the end of the tail (*d.*) to the marginal notch (*m.n.*). This condition was re-

garded by the author as evidence of concrescence. Considered in the light of the above facts, this view receives confirmation.

Owing to the enormously increased yolk, the germ-ring is cut off much earlier than in the forms represented in Fig. 3, having passed scarcely beyond the region of the equator.

With the increment of yolk there is a proportional increase in the extent of the primitive streak, until a certain point is reached, when it suffers reduction through the cutting off of the posterior portion of the embryo; so that in the Chick the anterior portion only of the primitive streak is present, the posterior portion being represented by the line extending from the tail-end of the embryo (*d.*) to the marginal notch (*m.n.*), plus the part represented by the rim of the blastopore. In the discussion of homologies it is all-important that this conception be kept in mind.

From a comparison of the above forms we observe that in Amphibia the greater portion of the embryo is formed through differentiation *in situ* and overgrowth, concrescence being confined to a limited region.

With the increase in yolk (most Teleosts) a greater extent (*p.s.*) of the embryo is formed by concrescence, the part arising through differentiation *in situ* and overgrowth undergoing a reduction. Through a still greater increment of yolk (certain Teleosts, Elasmobranchs, Aves) there is a corresponding increase in the extent formed by concrescence, while differentiation and overgrowth are of minor importance.

We conclude that the primitive method of embryo formation in vertebrates is through differentiation *in situ* and overgrowth, concrescence being a secondary method of uniting the lips of an elongated blastopore, this elongation having arisen through an increase of yolk.

The general remarks on the position and growth of the embryo, especially the Telostean and Avian, are largely the direct outcome of views expressed by Professor Whitman in his unpublished lectures on vertebrate embryology, and I gladly acknowledge my indebtedness to him.

10. THE NEURAL FOLDS IN AMBLYSTOMA.

The earliest stage in which the foundations of the neural folds are visible is represented in Pl. XX, Fig. 4. A transverse section shows slight lateral thickenings of the epiblast which, in a few hours, give rise to the bands (*n.f.*) shown in Fig. 5; these bands arise independently, and, so far as I am able to determine, differentiate *in situ*. They are later united anteriorly by a transverse thickening which forms the cephalic portion of the continuous fold. Clarke ('80) describes the folds as extending forward until they meet at the opposite end of the egg. Transverse markings such as shown in Pl. XXII, Fig. 9 (Necturus), are occasionally present in the neural plate at this time; to these, however, I give little emphasis, since I believe in most cases they are foldings between the myomeres, which at this time are beginning to form.

Pl. XX, Fig. 6, and Pl. XXII, Fig. 1, represent respectively the posterior and anterior portions of the same egg a few hours later. The neural bands have united to form a continuous fold, the cephalic portion having become especially prominent; the neural groove has appeared and extends to a point just within the cephalic fold. At the anterior end of the neural plate, on either side of the neural groove, is a slight depression, which in general outline is more or less circular, and in many cases pigmented; although by no means constant, their occasional presence is suggestive, since, as we shall see later in other forms, these areas form the bases of the paired eyes. A section of such an embryo is shown in Pl. XXII, Fig. 17: the foundation of the nervous system is a broad, thickened plate of epiblast, consisting of the epithelial (*ep.*) and sensory layers (*s.ep.*); beyond the region of the folds (*n.f.*), the sensory epiblast thins to a single layer of cells. Beneath the median portion of the neural plate there is a single layer of hypoblast (*hy.*) which forms the basis of the chorda (*ch.*). On either side of the chorda the sheets of so-called gastral mesoblast (*mes.*) are well defined, although not yet split into somatic and splanchnic layers. Just within the neural folds, on either side of the median line, are the depressions (*o.p.*) described above,

made up of narrow elongated cells which are deeply pigmented in their outer ends.

With the elongation of the embryo the neural folds become more prominent (Pl. XXII, Fig. 2). They are widely separated anteriorly, while posteriorly they are beginning to close. Certain markings which might be interpreted as neuromeres are often observed in the neural folds, yet their arrangement is decidedly irregular, and one is led to believe that they indicate nothing more than artifacts caused by the killing reagents.

The folds close more rapidly at the anterior end, so that in the succeeding stage (Fig. 3) they are about the same distance apart, throughout their entire length; in the cephalic region of the embryo, on either side there appear slight swellings. The anterior of these is the forerunner of the primary fore-brain, while the second represents the common foundation of the second and third cerebral vesicles. The folds soon close cephalad along their entire length. Occasionally I have seen the folds meet first at a middle point, as described by Clarke ('80).

In Fig. 4 the folds have closed, the outline of the embryo is well defined, the cerebral vesicles are distinct, the myomeres are differentiated throughout the greater portion of the embryo. The cranial nerves are forming; in short, the embryo is taking on those peculiarities which enable us to speak of it as a larva. I wish now to return to the consideration of certain features but faintly foreshadowed in the development of *Amblystoma*, namely:

II. THE OPTIC VESICLES.

Many attempts to explain the inverted position of the retina have led to the hypothesis that the vertebrate eye must have been located, originally, within the brain, Lankester ('80), Balfour ('85); or, as Beard ('88) stated: "Most of us now accept the view of Balfour, Carrière, and others, that the eyes were once structures opening dorsally on the surface of the unclosed neural plate."

While this was undoubtedly quite generally accepted, there were no observations to this effect until I showed in a prelimi-

nary paper ('93), the substance of which is here repeated, that this was precisely the case in *Rana palustris*. Soon afterward Locy ('93) found the same in the Shark.

It should not be said, however, that we were wholly without observations pointing toward an earlier differentiation. Bischoff, Kölliker, His, Van Beneden, and others have figured the early appearance of the optic vesicles in mammals. Heape ('84) finds in an early stage of the Mole, where the neural folds are closed along the center of the embryo, "at the anterior end the floor of the neural groove, on either side, is swollen, and on the outer and anterior edge of the two masses a deep narrow groove indicates the commencement of the formation of the optic organs."

Keibel ('89) describes a like condition in the embryo of the Guinea-pig. This apparently precocious development of the eyes in mammalia, showing no differentiation beyond the fact that depressions are present, is probably due to the retarded closure of the cephalic portion of the neural groove, and can scarcely be considered as a fact of phylogenetic significance.

Whitman ('89) discovered that in *Necturus* there is a very early appearance of the eye, "its basis being discernible as a circular area — after treatment with osmic acid followed by Merkel's fluid — long before the closure of the neural folds of the brain."

Through the kindness of Professor Whitman I have been able to study the development of the eyes in this form. In the stage (Fig. 10) corresponding closely to that described, the embryo is well defined, the closure of the neural folds having taken place posteriorly, and closely approximated well up toward the region which later forms the head. At the anterior end of the embryo, on either side, slight evaginations are visible. A section through these vesicles (Fig. 15), shows more numerous mitoses in these regions, as well as a marked migration of the nuclei toward the periphery. This is the earliest indication I have been able to make out satisfactorily. In Fig. 11 a later stage is represented, in which the optic vesicles are much better defined. Sections (Fig. 16) at this time show a continued migration of the nuclei, together with a thinning

of the wall until the optic vesicle is reduced to a single layer; meantime the external layer of the epiblast in this region has divided. In a few cases paired depressions have been found in earlier stages (Fig. 9). Sections, however, reveal nothing in the way of histological differentiation, and one might justly consider them as artifacts. The only thing which would dispel all doubt as to their meaning, would be to find some form in which they are so well marked that they may be traced, step by step, through the phases of involution of the neural plate, to the future optic vesicles. This condition is perfectly fulfilled by *Rana palustris*. In an early embryonic stage (Fig. 5), when the neural ridges are just forming, and are widely separated, paired depressions appear just within the cephalic folds, on either side of the median line. These areas are sharply contrasted with the surrounding parts by a much deeper pigmentation. A transverse section through them is shown in Fig. 12. The hypoblast (*hy.*) is a single layer forming the roof of the mesenteron. In the median line there is a fold which is the end of the chorda. In many cases this extends beyond the region of the neural fold, and is a point of theoretical interest. The mesoblast (*mes.*) consists of a single layer of cells, which passes insensibly into the axial region, where all three layers are fused. The sensory layer of the epiblast (*s.ep.*) exists as two lateral thickenings, united by the thinner median portion.

In *Amblystoma* and *Necturus* the superficial layer of the epiblast cannot be distinguished beyond the region of the neural folds, while in *Rana* it extends over the entire plate, as shown in the figure. In this layer the optic pits are formed. In addition to the fact that these areas are sharply defined by the presence of pigment to which more or less importance may be attached, they are further remarkable in that the elongation of the cells and the position of nuclei are indicative of a considerable degree of histological differentiation. Between these areas the cells are undifferentiated and resemble those of the superficial epiblast in other parts of the embryo. Sections further posterior show the cells of this layer to be uniform. Fig. 13 represents a section through a later stage, when the folds are still

widely separated. There is but little change in the histological character of the cells, except that their boundaries are less distinct. Owing to division and loss of yolk, the cells of the embryo have gradually become smaller and more compact. The originally broad space enclosed between the neural folds has undergone a constant reduction in size; a rapid closing of the anterior portion has taken place, so that along the entire length of the embryo the folds are approximated.

A section at this stage is represented in Fig. 14. The canal is elliptical owing to the slight evagination of the optic areas which are the forerunners of the optic vesicles. Their bilaterality is indicated by the distribution of pigment only, and one might justly consider them as derived from a common basis. A marked migration of the pigment has taken place; instead of being located at the ends of the cells as in earlier stages, it is found between them and nearer the periphery. The nuclei have likewise undergone a further migration toward the surface, so that the cells of the superficial layer have completely lost their identity.

It is of interest to note that in *Petromyzon* at a stage corresponding closely to the above, Kupffer ('90) has observed that an unpaired basis for the eyes is present as the following quotations show: "In der Mittelebene zeigt sich gar kein Merkzeichen welches auf Duplicität der Anlage deutet, dieselbe ist vielmehr zunächst eine unpaarige. . . . Die später paarige Erscheinung des Organs wird nur darin angedeutet, dass in den lateralen Regionen der Erweiterung beiderseits Mitosen auftreten, die am Boden fehlen."

During the later stages of development the walls of the vesicles become thinner so that just before they invaginate to form the optic cups they consist of but a single layer of elongated cells with their nuclei located in their peripheral ends. A continued dispersion and migration of the pigment takes place, and at this time is more abundant in the stalk than in the portion which will later form the optic cup. The later development of the vesicles in this form is essentially the same as in *Necturus* and *Amblystoma*. Since these stages have been carefully studied by Professor Mall ('93) a further description would be superfluous.

12. THE OLFACTORY AND ORAL GROOVES IN RANA.

In Pl. XXII, Fig. 6, an embryo of 70 hrs. is represented. The neural folds are anteriorly wide apart. At this stage paired grooves (*ol.g.*) arise in the cephalic portion of the neural fold; these grooves are short, shallow and deeply pigmented.

A later stage is represented in Fig. 7 in which the neural folds are united along their entire length. The distal ends of the grooves have extended antero-laterally, while the proximal ends have been united by the closure of the neural folds causing the V-shaped arrangement shown in the drawing.

In Fig. 8, the grooves have reached their maximum extension, having grown antero-laterally until their distal ends reach well over toward the anterior border of the primary fore-brain. At this time the pits form at the distal ends of the grooves and the nose becomes localized. The lines of pigment are exactly similar to, and continuous with, the line which indicates the suture formed by the closure of the neural folds.

The mouth usually arises as an independent median invagination; in some cases, however, it shows extremely interesting peculiarities.

In the development of the nervous system of *Rana* the closure of the neural folds occurs in two ways; the most frequent is by a median approximation of the lateral portions of the neural folds, together with an antero-posterior folding of the cephalic portion. Often the antero-posterior folding does not occur, or if so, to a slight extent only. When this occurs a very shallow pigmented groove is formed just at the anterior end of the neural groove.

At a time shortly after the folds have begun to approach posteriorly the appearance of the oval groove is foretold by a very slight depression at the anterior end of the neural groove. This would appear in a somewhat earlier stage than Fig. 6. With the progressive closure the groove extends farther anteriorly (Figs. 7 and 8); in the latter it reaches just beyond the region of the primary fore-brain. The oval invagination is now outlined and localized in the distal end of the groove in a manner quite similar to the olfactory pits.

Concerning the significance of these structures it can only be said that from our knowledge of the organ in other groups we should scarcely be warranted in considering them of phylogenetic importance; yet it cannot be said that these peculiarities are without hypothetical interest.

13. PARAPHYSIS AND EPIPHYSIS IN AMBLYSTOMA.

From the numerous late researches it seems probable that in most forms there are at least two median outgrowths in the roof of the encephalon.

The posterior of these, the epiphysis, arises according to the majority of observers, among whom I may name Béraneck ('87), Strahl and Martin ('88), Françotte ('88), Selenka ('90), and Klinckowstrom ('93), as a median unpaired evagination in the roof of the thalamencephalon; this structure soon gives rise to a secondary, the parietal vesicle or pineal eye.

Others hold that instead of considering one of these vesicles as secondary they are to be considered as the representatives of two homodynamous structures, Leydig ('90), Hill ('91), Locy ('93).

Again there is found a group of several vesicles arising in this region, Duval and Kalt, Prenant, and others.

The anterior, or paraphysis, generally arises slightly anterior to the line which marks the boundary between prosencephalon and thalamencephalon. Concerning the fate and function of this organ there is much difference of opinion. Françotte ('88) derives from it a portion of the future choroid plexus. Selenka ('90) suggests that it may be the rudiment of an ancestral ear, while Leydig ('90) finds, instead of a single vesicle, a group of five which later come into such close relation to the epiphysis that Spencer ('84) figured both as one structure.

In a brief preliminary ('92) I showed the epiphysis and paraphysis to be entirely independent structures in *Amblystoma*. In the following pages I repeat the principal facts:

The first trace of the epiphysis is in a 5 mm. embryo where it shows, in section, as a crescentic evagination in the roof of the thalamencephalon. The lateral walls are formed of several layers of cells, while the dorsal, which comes directly in con-

tact with the superficial layer of the epiblast is but a single layer. The nuclei undergo a marked migration toward the periphery, and in this respect the appearance is strikingly similar to the condition found in the optic vesicles, which at this time are strongly evaginated. The presence of pigment at the inner ends of the cells is also a significant fact.

From this time until the formation of the lens in the lateral eye, the epiphysis increases in size, its cavity becomes elliptical and is in wide communication with the thalamocoele.

At the time of the invagination of the lens there appears in the posterior portion of the roof of the prosencephalon a second median outgrowth which is probably homologous with the paraphysis described in Reptilia by Selenka.

In a 12 mm. larva the paraphysis is much elongated; lateral diverticula appear at its distal end while the cavity is obliterated proximally in a manner analogous to that which occurs in the epiphysis. The changes are more pronounced in 14 mm. larva where it has assumed a digitate appearance and bears a striking resemblance to the true choroid plexus of the lateral ventricles. Hill ('94) has described the paraphysis in *Amia* and believes that it likewise later forms a part of the choroid plexus.

The two structures in Urodela never come into close relation, as in Reptilia, but remain widely separated. That the paraphysis is indeed a peculiar organ, very similar in development to the epiphysis, is shown not only by Selenka but also by Leydig in his final memoirs, where from its exceptional mode of development, the name "anterior epiphysis" is suggested. The hypothesis of Selenka that it is a degenerate sense organ, is certainly questionable; much more that its ancestral function was audition.

The variation in the point of origin of the paraphysis, and its formation at a much later period than the epiphysis, would seem to indicate a less important ancestral function. The phylogenetic importance of the epiphysis is certainly indicated by the fact that it is formed at a fixed point throughout the vertebrate phylum. While it must be admitted that we are without evidence sufficient to warrant us in making any

very definite statement, since if the organ is rudimentary its development may be retarded, yet its ontogeny indicates that it arose at a time when a neural canal was first formed. If we admit the hypothesis, which I believe I have proved, namely, that the lateral eyes are present as a pair of depressions in the cephalic neural plate, we might anticipate that at the phylogenetic period when they become implicated by the closure of the neural folds a median eye would arise and become most highly functional during the period when the lateral eyes were non- or least functional. This might explain the origin of the epiphysis as an unpaired organ; the circumstances which give rise to another sense organ, if such the paraphysis be considered, are extremely difficult to explain. In my preliminary note I suggested the above hypothesis as one of the possible explanations of the origin of a median unpaired visual organ.

Concerning this hypothesis Professor Béraneck ('92) says:

"Quel a été le rôle de l'œil pariétal? Pourquoi s'est-il développé? Eycleshymer cherche à résoudre ce problème en disant que, lors de la fermeture du tube médullaire, les yeux pairs — auparavant directement influencés par la lumière — perdirent d'une manière plus ou moins complète leur fonction. Cette perte d'activité fonctionnelle fut compensée par l'apparition d'un œil dorsal impair, qui commença à s'atrophier, lorsque les yeux pairs eurent reconquis leur prépondérance.

"Cette explication ne me satisfait pas, je l'avoue. Tout d'abord, j'ai peine à comprendre que les yeux pairs perdent leurs fonctions pour les reprendre plus tard, alors qu'un autre organe visuel est apparu entretemps pour les suppléer. Puis ce rôle de suppléant, joué par l'œil pariétal, s'accorde peu avec la persistance de cet organe dans un groupe de Vertébrés aussi élevé que celui des Sauriens, groupe dont les yeux pairs sont fort bien organisés."

Although laying but little stress upon this view, as expressly stated in my preliminary, I still maintain that there is nothing unphysiological in postulating the decrease of function in a given organ for a certain length of time and its gradual revival under favorable environment. Lankester ('80) and Balfour

('85) have both expressed such views concerning the development of the paired eyes.

The fact that Béranek, Strahl and Martin, Françotte, Prenant, and others have found a transitory nerve passing to the pineal organ, might even be considered additional evidence of its having been a supplementary organ. Why it should have continued functional in some forms as indicated by a rich nerve supply, and in others degenerated, is an entirely secondary question.

The origin of the pineal organ, in the manner described by Leydig and Hill may indicate that it is derived from paired sensory areas. The accessory vesicles described by Locy are also very suggestive, and later investigation may prove that the parietal eye arises from the coalescence of two such structures. The recent work of Kupffer, however, on the origin of the secondary vesicle in *Petromyzon*, in which form the structure is functional, if we may judge from the rich nerve supply, shows nothing which can be interpreted as indicating a paired origin.

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<i>a.</i>	Anus.	<i>n.f.</i>	Neural folds.
<i>bp.</i>	Blastopore.	<i>ng.</i>	Neural groove.
<i>ch.</i>	Chorda.	<i>np.</i>	Neuropore.
<i>ck.</i>	Caudal knob.	<i>og.</i>	Oral groove.
<i>cv.</i>	Caudal vesicle.	<i>olg.</i>	Olfactory groove.
<i>cv'.</i>	Secondary caudal vesicle.	<i>op.</i>	Optic pits.
<i>dl.</i>	Dorsal lip of blastopore.	<i>per.</i>	Periblast.
<i>ep.</i>	Epiblast.	<i>s.c.</i>	Segmentation cavity.
<i>g.c.</i>	Gastral cavity.	<i>s.ep.</i>	Sensory epiblast.
<i>gr.</i>	Germ-ring.	<i>tel.</i>	Teloblast.
<i>hy.</i>	Hypoblast.	<i>v.p.</i>	Vegetative pole.
<i>K.v.</i>	Kupffer's vesicle.	<i>yk.</i>	Yolk.
<i>m.</i>	Mesoblast anlage.	<i>yp.</i>	Yolk plug.
<i>mes.</i>	Mesoblast.		

EXPLANATION OF PLATE XVIII.

All figures from Amblystoma punctatum.

FIG. 1. Egg with enclosing membranes just after laying ($\times 8$).

FIG. 2. Superior hemisphere of egg 2 hrs. after laying, showing "light spot," within which lies the first polar body ($\times 15$).

FIG. 3. Superior hemisphere 4 hrs. after laying, showing eccentric aggregation of pigment, also presence of second polar body ($\times 15$).

FIGS. 4-12. Successive stages of cleavage from living egg ($\times 15$).

FIGS. 13-18. Successive stages of cleavage in superior hemisphere of living egg, showing order and time of appearance ($\times 15$). Heavy black line indicates the first cleavage; heavy black broken line, second cleavage; blue line, third cleavage; heavy red line, fourth cleavage; black dotted line, fifth cleavage; red dotted line, sixth cleavage; light black line, seventh cleavage. Numerals indicate time of first appearance of the furrows.

FIG. 19. Meridional section of egg in stage corresponding to Fig. 7, showing early segmentation cavity ($\times 20$).

FIG. 20. Meridional section through the centers of least and most densely pigmented areas in stage corresponding to Fig. 9 ($\times 30$).

FIGS. 21, 22. Meridional sections through same areas, showing thickening of roof in densely pigmented area, and thinning at opposite side ($\times 35, 40$).

1.



2.

c

3.

4.



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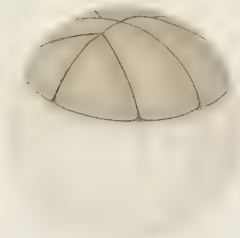
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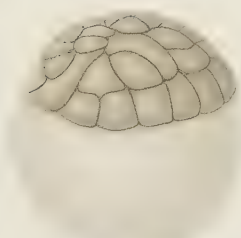
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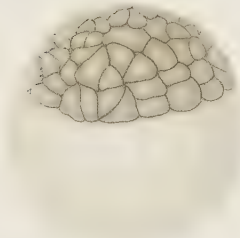
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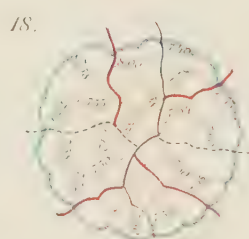
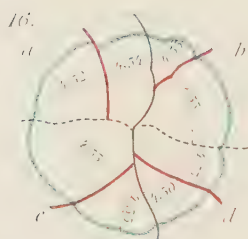
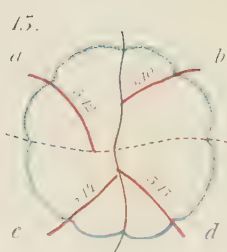
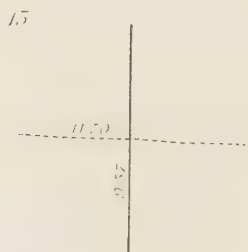


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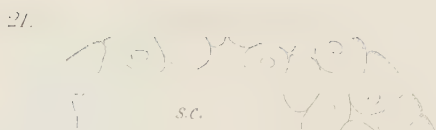
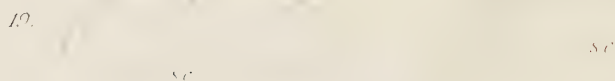


12.





22.



EXPLANATION OF PLATE XIX.

FIGS. 1-15. Cleavage in superior hemisphere of egg of *Petromyzon marinus* ($\times 30$).

Figs. 1-4. Successive stages in cleavage of living egg.

Figs. 5, 6, 7. Successive stages in cleavage of second living egg.

Figs. 8, 9. Variations in formation of first and second sets of furrows.

Figs. 10-13. Variations in formation of third set of furrows.

Figs. 14, 15. Later cleavage.

FIGS. 16-35. Cleavage of *Coregonus albus* ($\times 15$).

Figs. 16, 17, 18, 19. Variations in first cleavage.

Figs. 20, 21. Three blastomeres arising from unequal two-cell stage.

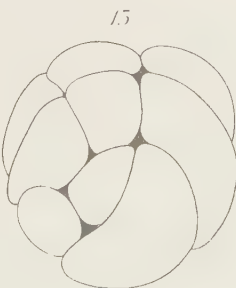
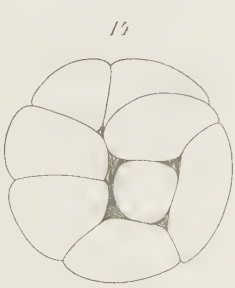
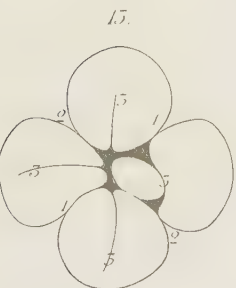
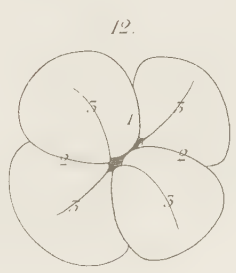
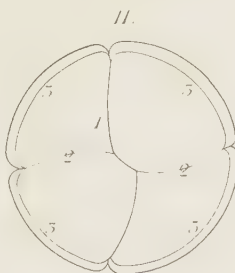
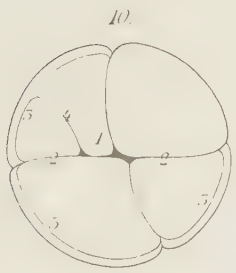
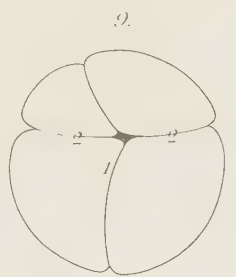
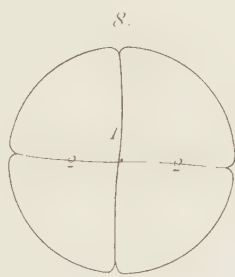
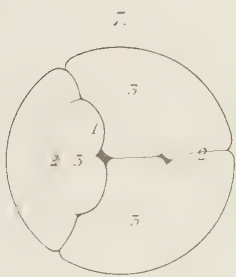
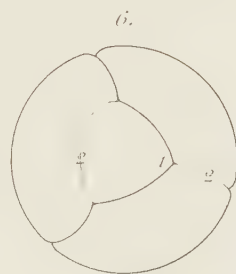
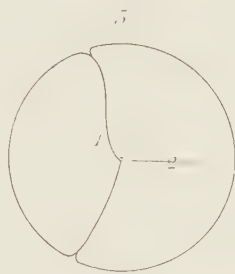
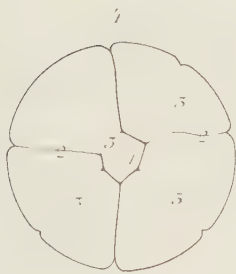
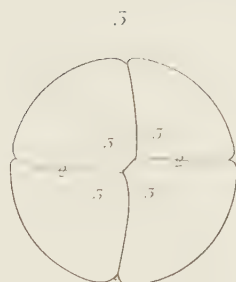
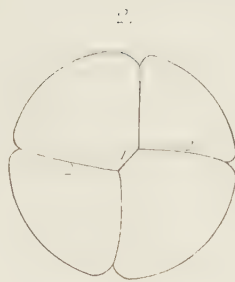
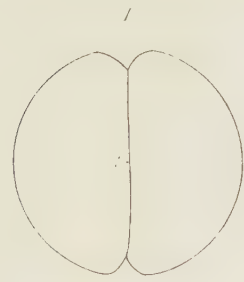
Figs. 22, 23, 24. Variations in second cleavage.

Figs. 25, 26. Conditions derived from unequal two-cell stage.

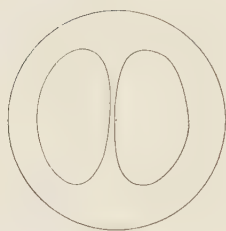
Figs. 27, 28, 29, 30, 31. Variations in third cleavage.

Figs. 32, 33. Fourth cleavage.

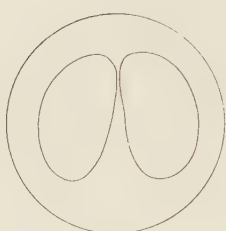
Figs. 34, 35. Later cleavage stages.



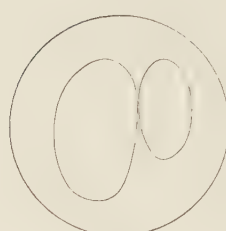
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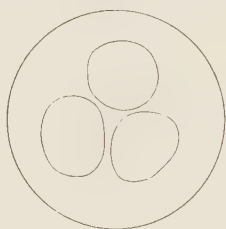
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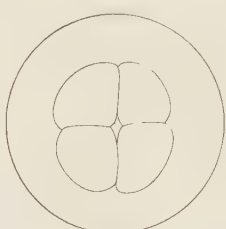
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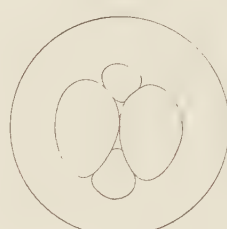
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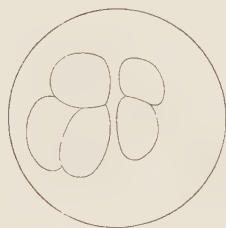
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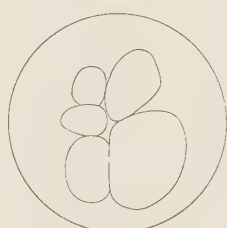
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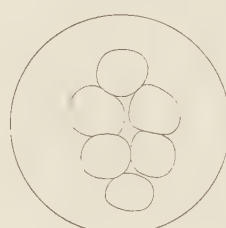
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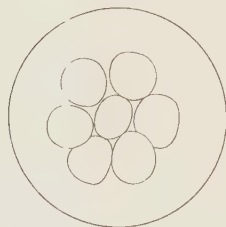
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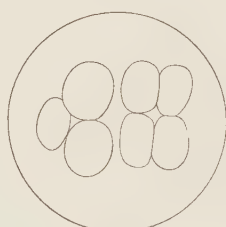
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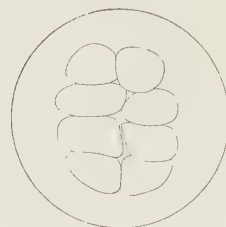
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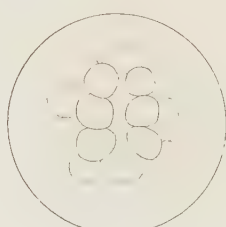
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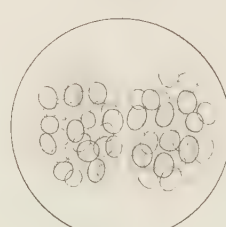
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34.



35.



EXPLANATION OF PLATE XX.

FIGS. 1-6. Surface views of successive stages in the formation of the embryo of *Amblystoma punctatum* ($\times 15$).

FIG. 1. Surface view, showing irregular depression among yolk cells at vegetative pole, also first indication of blastopore ($\times 15$).

Figs. 2, 3. Surface views of postero-ventral surface, showing later stages in extension of blastopore ($\times 15$).

Figs. 4, 5, 6. Surface views of postero-ventral portion, showing successive stages in closure of blastopore, also the formation of neural folds ($\times 15$).

FIG. 7. Ventral surface view of extremely large blastopore ($\times 15$).

FIGS. 8, 9. Surface views of blastopore of *Rana palustris* ($\times 15$).

FIG. 10. Surface view of *Petromyzon marinus*, drawn from living egg by Dr. Johnson, showing lateral extension of the blastopore ($\times 30$).

FIGS. 11-14. Surface views of *Amiurus catus*, showing formation of embryo ($\times 15$).

FIG. 15. Shows portion of germ-ring left behind the embryo.

FIG. 16. Surface view of *Coregonus albus*, showing late stage in closure of blastopore ($\times 15$).

FIGS. 17-24. Successive stages in the formation of the embryo of *Lophius piscatorius*, showing the part of the germ-ring left behind ($\times 45$).



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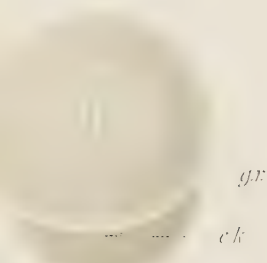
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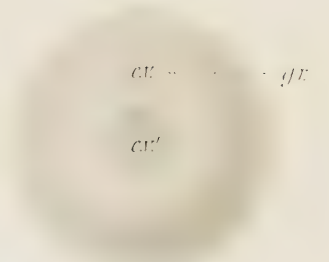
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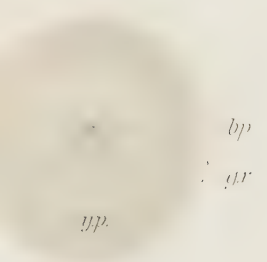
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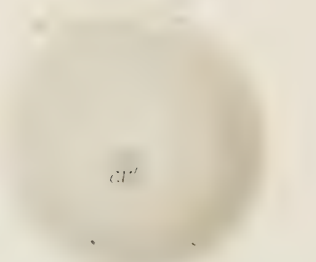
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24.



EXPLANATION OF PLATE XXI.

- FIG. 1. Meridional section of egg at stage shown in Fig. 7, Pl. XX ($\times 45$).
FIG. 2. Section along line *xy* of Fig. 2, Pl. XX ($\times 45$).
FIG. 3. Section along line *xy* of Fig. 3, Pl. XX ($\times 45$).
FIG. 4. Saggital section of a stage about the same as indicated in Fig. 4, Pl. XX ($\times 45$).
FIG. 5. Saggital section of egg of *Petromyzon marinus* at a stage indicated by Fig. 10, Pl. XX ($\times 90$).
FIG. 6. Saggital section of gastrula of *Petromyzon marinus*, little later than Fig. 10, Pl. XX.
FIG. 7. Saggital section of *Petromyzon marinus* after Hatta.
FIG. 8. Saggital section of *Petromyzon marinus* showing teloblast.
FIG. 9. Transverse section of *Amblystoma*, at stage intermediate between Figs. 4 and 5, Pl. XX ($\times 90$).
FIG. 10. Section through caudal vesicle of *Lophius* at stage shown in Fig. 24, Pl. XX ($\times 100$).
FIG. 11. Portion of 10 marked (*per.*) more highly magnified.
FIG. 12. Section across post-embryonic portion of germ-ring of *Amiurus* at a stage indicated by Fig. 15, Pl. XX ($\times 300$).
FIG. 13. Saggital section through posterior portion of embryo of *Lophius* at stage indicated by Fig. 21, Pl. XX ($\times 250$).
FIG. 14. Saggital section through posterior portion of embryo of *Lophius* at stage indicated by Fig. 22, Pl. XX ($\times 250$).
FIG. 15. Saggital section through same region at a later stage ($\times 250$).

EXPLANATION OF PLATE XXII.

FIGS. 1-4. Successive phases in closure of neural folds of *Amblystoma* ($\times 15$).

FIGS. 5-8. Successive phases in closure of neural folds of *Rana palustris* showing the infolding of the optic pits, and the formation and extension of the oral and olfactory grooves ($\times 15$).

FIGS. 9-11. Successive phases in closure of neural folds of *Necturus*, showing formation of optic vesicles ($\times 10$).

FIG. 12. Transverse section through dark areas shown in Fig. 5 ($\times 120$).

FIG. 13. Transverse section through same areas as shown in Fig. 6 ($\times 130$).

FIG. 14. Transverse section through same areas as shown in Fig. 7 ($\times 100$).

FIG. 15. Transverse section along line 15 in Fig. 10 ($\times 90$).

FIG. 16. Transverse section along line 16 in Fig. 11 ($\times 80$).

FIG. 17. Transverse section along line 17 in Fig. 1 ($\times 100$).

